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CONTENTS

	PAGE
THE INTERMEDIARY METABOLISM OF FRUCTOSE AND GALACTOSE. *Harry J. Deuel, Jr.*	173
ELECTRICAL PHENOMENA IN LARGE PLANT CELLS. *W. J. V. Osterhout*	216
VITAMIN C, ASCORBIC ACID. *C. G. King*	238
THE ECONOMY OF MUSCULAR EXERCISE. *D. B. Dill*	263
THE PHYSIOLOGICAL ACTIVITY OF NUCLEIC ACID AND ITS DERIVATIVES. *Alan N. Drury*	292

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PHYSIOLOGICAL REVIEWS

VOL. 16 APRIL, 1936 No. 2

THE INTERMEDIARY METABOLISM OF FRUCTOSE AND GALACTOSE

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Although there can be no doubt that the ingested fructose or galactose is capable of being transformed to glucose in the animal body, one cannot question the individuality in the behavior of each of the hexoses. If one accept the more or less current opinion that the metabolism of fructose and galactose follows a primary course which results in their quantitative conversion to glucose after which they are metabolized in a manner identical with the last sugar, then the only difference which one should observe in the intermediary metabolism of the three sugars should be traceable to their variability in rate of absorption and the speed with which they become available as glucose.

However, there are a number of facts which lead one to question the validity of the above hypothesis. For example, the rate of glycogen deposition after fructose and galactose is not identical with their capability of forming glucose. Also their speed of oxidation, the specific dynamic action which they produce and their effect on the respiratory quotient are all entirely out of line with their rate of change to glucose. Experiments on their ketolytic activity show that glucose is the least effective of the three. The lactic acid formation following the metabolism of the three hexoses in question is variable. All these facts lead one at least to inquire whether oxidation or glycogen formation may not precede glucose formation. Another suggestion is that fat formation may take place more readily from one hexose than from another.

Glucose has always been considered the physiological sugar—largely because it is present in the blood and also since it is the end product arising from the enzymatic or acid hydrolysis of glycogen. On the other hand, a sugar like galactose which is an essential component of nervous tissue, must also be classed as a physiological carbohydrate

rather than a "foreign" sugar. There is every reason for believing that this sugar can be synthesized in the male as well as in the lactating female to supply the needs for building up the galacto-lipids. Fructose must also be considered as a physiological hexose. Although it is not known to be a component of tissues, its ready formation from glucose diphosphoric acid in the muscle tissues is an indication of its ready synthesis as well as its importance in the process of glycolysis.

In the present review, the fate of fructose and galactose has been compared with that of glucose through the various stages in their intermediary metabolism. Absorption, transportation in the blood, glycogen formation, oxidation, ketolysis and tolerance have each been considered in turn. The site of transformation to glucose as well as the action of insulin on various processes have been discussed.

ABSORPTION OF SUGARS FROM THE GASTRO-INTESTINAL TRACT. Galactose and fructose appear to be absorbed at least partially from the small intestine in unchanged form. The concentration in which they appear in the blood is influenced by the rate of absorption, by the rapidity with which they are converted to glucose or glycogen, as well as by the speed with which they are oxidized or converted to fat. If the liver is unable to remove the monosaccharide as rapidly as absorption takes place, the unchanged sugar is found in the systemic as well as in the portal blood and a melituria may take place.

The rate of absorption of the monosaccharides from the gastro-intestinal tract is probably not identical even when administered in uniform concentrations. It is generally considered that galactose is absorbed somewhat faster and fructose considerably slower than is glucose. Such findings were reported by Nagano (1902) on dogs with a Vella fistula and by Cori (1925) with rats employing a technique devised by him. Similar results on rats were found by Wilbrandt and Laszt (1933), Wertheimer (1933), Westenbrink (1934), and Verzar (1935). Confirmation of the slower rate of absorption of fructose is found in the tests of Hewitt (1924) and Hedon (1900) on rabbits. The comparative values obtained by Cori were 110 for galactose, 100 for glucose and 43 for fructose.

Contradictory evidence on the relative rate of absorption of galactose was obtained by Hewitt (1924) who showed that this hexose was absorbed more slowly than glucose in narcotized cats. Cori believes that these results may be attributed to a species difference. Emslie and Henry (1932) also noted that galactose was absorbed somewhat more slowly by chickens than was glucose. Cajori and Karr (1935) report no variation in the rate of absorption of galactose and glucose from the

Thiry loops of the jejunum in dogs. Burget, Moore, and Lloyd (1932a) in an extensive series of experiments on the absorption of glucose and fructose with dogs having a closed loop of intestine, with rabbits having an ileal loop, and with rats employing the Cori technique have found that the difference in their rate of absorption is much less than that reported by Cori. Fructose was absorbed only 9 per cent less rapidly by rats than was glucose, 5 per cent less with rabbits and from 4 to 11 per cent slower with dogs. Shaffer and Somogyi (1933) have criticized the procedure of Burget, Moore, and Lloyd in determining sugar on the untreated intestinal contents. Moreover, they have failed to take into consideration the body weight of the rats.

Although the majority of data seem to indicate that the monosaccharides are absorbed in the order which was reported by Cori (1925), some of the experimental results are sufficiently conflicting to make a further study advisable. In all probability, hitherto unrecognized factors are responsible for the divergent results obtained by the different investigators. In such work it is necessary particularly to avoid such complicating factors as the retention of sugar solution in the stomach, and the data should be sufficiently extensive to be capable of statistical analysis.

The speed of absorption of sugar has been found to be independent of the absolute amount of sugar present even after 70 per cent of the original sugar has been absorbed (Cori, 1925). Moreover, it was noted that 25, 50, and 80 per cent glucose solutions were assimilated at identical rates which would seem to support further the previous results. Trimble, Carey, and Maddock (1933) confirm Cori's results in experiments on dogs in which varying concentrations of glucose were introduced. In a later paper, Trimble and Maddock (1934) report additional experimental evidence in support of their contention of the uniformity of glucose absorption which amounted to 0.92 gram per kilo per hour. MacKay and Bergman (1933) have failed to confirm Cori's results on rats and report a considerably higher absorption rate the first hour which may reach an essentially constant level beginning with the second or third hour. The amount of glucose absorbed per unit body surface (the absorption coefficient) was found much more uniform in different animals than when the amount of absorption was based on body weight. These authors further criticize the interpretations of the results of Trimble et al. (1933) because the dose of glucose administered to dogs killed after longer periods was increased over that administered for their shorter experiments. The experiments of MacKay and Bergman are further discussed by the Coris (1934). Maddock,

Trimble, and Carey (1933) demonstrate that no absorption of glucose takes place in the stomach of the dog. Even, with larger doses of this sugar as employed in the longer tests of Trimble et al (1933), the quantity of glucose introduced into the stomach did not necessarily increase the amount available for absorption in the gut since much of it remained in the stomach. Verzar (1935) found that solutions of glucose and galactose varying in concentration from 2.7 to 12.8 per cent were absorbed at uniform rate in rats. With fructose an increasing speed of absorption occurs with higher concentrations. The variation in absorption of the latter sugar may be because it is changed in the gut. Pierce, Osgood, and Polansky (1928–29) found that the rate of absorption of glucose was not uniform in rats although the later periods closely approximate those of Cori (1925). In addition Burget, Moore and Lloyd (1932) showed that a considerable decrease in the rate of absorption of both glucose and fructose obtained during the second hour in experiments on rats. The maximum rate of absorption took place during the first 30 minute period. Obviously, further experimental evidence on the effect of concentration of various sugars on their relative absorption rates is needed.

The rate of absorption of galactose apparently is influenced to a large extent by the presence of other sugars in the intestine. Folin and Berglund (1922) demonstrated the fact that the addition of 100 grams of glucose to a meal of 100 grams of galactose reduced the sugar excretion to one-tenth of the original level obtained when galactose was taken alone. They suggested that the extent to which galactose could be metabolized was dependent on the quantity of glucose available. Similar results were obtained on dogs by Bodansky (1923). Corley (1927b) could demonstrate no difference in the galactosuria obtained in rabbits when galactose or galactose plus glucose was administered intravenously; after oral administration, however, the latter investigator obtained a confirmation of the results of Folin and Berglund (Corley, 1928). Fructose did not possess the ability of glucose to lower the galactosuria (Bodansky, 1923; Corley, 1928). Harding and Grant (1932–33) demonstrated a marked drop in galactosuria when 30 grams of glucose were added to a standard test meal of 40 grams of galactose. Moreover, the galactemia was markedly reduced and reached a much lower peak. All of these observations are explained on the basis of the experiments of Cori and Cori (1927–28) on rats which demonstrated that the rate of absorption of galactose from the intestine was markedly reduced concomitantly with a decrease in the galactosuria if glucose also was administered. The best utilization occurred when the galac-

tose was given in the form of lactose. The rate of absorption of galactose from 1, galactose alone, 2, galactose plus glucose, and 3, lactose was 100:41:15 respectively while the comparison in utilization was of the order 100:153:240. A similar retardation in absorption of galactose from Thiry loops of jejunum of dogs was noted by Cajori and Karr (1935) when glucose was added.

Magee and Reid (1932) and Wilbrant and Laszt (1933) have reported that the absorption of hexoses first involves the formation of phosphoric acid esters, which supports the earlier results of Abelin and Goldener (1925). Laszt (1935a) found that phosphate buffers augmented the absorption of glucose only at a pH of 7.0 in tests on rats. That the phosphate ion was not specific is indicated by the fact that increased absorption of glucose also occurred at the same pH with borate or acetate buffers. Laszt (1935b) confirmed his previous work by demonstrating that glycerol extracts of the intestinal mucosa of rats caused a decrease in inorganic phosphate when glucose, galactose or fructose were present while negative results were given by mannose and xylose. Sodium mono-iodoacetate prevented the conjugation of phosphoric acid and the hexoses; this substance also prevented an increased rate of absorption in *in vivo* experiments when phosphates were added. The enzymatic effect of the intestinal extract was destroyed by heating to 52–65°C. Mathieu (1935), however, showed that the Harden-Young, Robison and Neuberg esters were all absorbed more slowly than glucose alone. Moreover, Cajori and Karr (1935) were unable to demonstrate any effect of phosphates in the gut on the rate of absorption of the hexoses thereform; sulfates and chlorides likewise were without such activity.

Different rates of absorption of the hexoses were also shown to obtain after subcutaneous administration to rats by Himwich, Goldman and Krosnick (1932). Approximately 48.0 per cent on an average of administered glucose had been removed from the site of injection at the end of 45 minutes while 39.2 per cent of galactose and 27.0 per cent of fructose were absorbed in the same period. An apparently constant rate was noted for a concentrated solution (50 per cent) while a diminishing value was obtained for dilute solutions (5 and 2.5 per cent). Cori and Goltz (1925–26) noted that galactose and fructose (as well as other monosaccharides) were absorbed from the peritoneal cavity at an identical rate with glucose. The variation in rates of absorption from the peritoneal cavity and the intestines is ascribed by them to the different epithelial lining of these two membranes.

Kozuka (1926) also indicated that fructose may be absorbed by way of the lymphatics. Fay and Wharton (1935) have indicated that the proportion of non-fermentable reducing substances carried in the lymph of dogs is practically identical with the amount transported in the blood after the administration of galactose. Average values were 9.1 mgm. per cent.

EFFECT ON THE BLOOD SUGAR LEVEL. That fructose and galactose may enter the blood stream in unaltered form is evident from the fact that the excretion of the unchanged sugars may occur in the urine when large doses of them are taken. Such results could only be obtained if the carbohydrates were carried in unchanged form from the gut to the kidney.

Proof of the presence of fructose and galactose in the blood stream has been recently brought forward with the development of more refined technique for the determination of small amounts of the specific carbohydrates. The effect of the monosaccharides on the blood stream cannot be interpreted from much of the earlier work because the concentration of the different sugars in the blood was not differentiated but only the total reducing value was noted. In view of the fact that fructose and galactose reduce such copper solutions to a different extent than glucose it is evident that values for the total reducing power of the blood are inaccurate if monosaccharides other than glucose are also present in the blood. Moreover, such information does not indicate whether the rise was caused by the specific sugar fed or whether it is to be traced to a concomitant rise in the glucose content in the blood.

Determination of fructose in blood. Most of the earlier determinations involve an attempt to apply the qualitative Seliwanoff (1887) reaction to blood. Such procedures were used by Folin and Berglund (1922) and by Kronenberger and Radt (1927). von Crevald (1927) employed the blue color produced when a mercuric chloride filtrate of serum containing fructose was heated with diphenylamine and hydrochloric acid, for its colorimetric estimation. This procedure was capable of detecting fructose in a concentration of 0.001 per cent. This latter procedure was satisfactorily modified by Radt (1928). Corley (1929) altered the technique to make it suitable for tungstic acid filtrates as well as urine or aqueous solutions of this sugar. Glucose, xylose, d- and l-arabinose and galactose caused only 3 per cent of the color development of fructose. Burget, Moore and Lloyd (1932b) report this method accurate to 0.005 per cent.

Campbell and Hanna (1926) employed a method based on the reduc-

tion of molybdenum with potassium permanganate. The method is somewhat unsatisfactory because it is not entirely specific or very sensitive and requires considerable time for its completion. Bollman and Mann (1931), however, seem to have employed it satisfactorily. Harding, Nicholson, and Armstrong (1933) were able to differentiate glucose from fructose and mannose by the use of a special strain of *Proteus vulgaris*. This method is satisfactory for small amounts of blood. Most recently, Roe (1934) has modified the resorcinol–HCl reaction for fructose by increasing the HCl from 12 to 18 per cent and adding ethyl alcohol as a solvent for the red-colored substance produced. The interference with glucose is avoided by reduction at a temperature of 80°C. Such a procedure can be employed satisfactorily for colorimetric comparisons.

It seems probable that the procedure employed by Deuel and Chambers (1925) for differentiating fructose and glucose in the urine might profitably be used for their separate determination in blood. This method makes use of the fact that fructose is completely destroyed by refluxing with 10 per cent HCl while glucose is not affected. Kruisheer (1929) has made use of this procedure in the determination of fructose and glucose by titration after treatment with alkaline iodide solution.

Factors altering fructose concentration in the blood. All experimental evidence points to the fact that the rise in blood sugar after the administration of fructose is much less than that caused by an equal amount of glucose (Folin and Berglund, 1922; Bodansky, 1923; Reinhold and Karr, 1927; Bertram, 1929, and others). That such a hyperglycemia is caused at least in part by the presence of fructose in the blood is indicated by the experiments of Folin and Berglund (1922); Kronenberger and Radt (1927); Oppel (1929); Corley (1929); Harding, Nicholson, and Armstrong (1933) and others.

Folin and Berglund (1922) have explained the failure of fructose to cause a greater rise in blood sugar as due to its rapid absorption from the blood by the tissues. Because of the relative "saturation" for glucose, a less ready removal obtains for the latter sugar. The marked hyperglycemia after galactose in spite of the absence of unsaturation of this sugar in the tissues would seem to make such a theory untenable. Cori (1925) believes that the lower glycemia of fructose is largely to be traced to its slower rate of absorption from the gut. The observations of Reinhold and Karr (1927) indicate that the low level of blood sugar after fructose is also to be attributed to its more rapid removal from the blood for glycogen formation and possibly because of its readier oxida-

tion. When the latter conditions did not obtain (as in the experiments with 4 day fasted rabbits), the blood sugar rose to a higher level after 6 grams of fructose per kilo than after a similar dose of glucose. As further support both of the theories of Cori and of Reinhold and Karr, Jolliffe (1930–31) postulates that glycogenesis is stimulated when the blood sugar reaches a level between 95 and 110 mgm. Higher blood sugar values obtain after the administration of rapidly absorbed hexoses as galactose because glycogenesis does not keep pace with absorption. With slowly absorbed sugars such as fructose, glycogenesis proceeds practically as rapidly as absorption with the result that the "excitatory ceiling" is overshot only a little.

After the administration of 10 grams of fructose to normal rabbits, Corley (1929) noted that the fructose appeared in the blood shortly after feeding, reached a maximum level in about an hour, and practically disappeared during the second hour. The presence of fructose did not always cause a rise in total blood sugar. In normal animals the fructose concentration at its maximum did not account for more than 15 per cent of the total sugar. The course of the fructose curve in the blood was uninfluenced by the administration of phlorhizin. After intravenous doses of 2 grams per kilogram, fructose appeared in large amount in the blood but disappeared within 90 minutes. The rate of its disappearance in such cases was altered appreciably only after excessive doses of liver poisons. Oppel (1929) noted the presence of fructose in the blood within 5 minutes after the oral administration of this sugar in doses of 2.0 to 3.5 grams per kilogram to rabbits. Maximum values for levulose of 8 to 10 mgm. per cent were noted in the portal blood. The levulosemia persisted as long as 3 hours after this dose of sugar. In fructose tolerance tests carried out on 92 subjects, Jolliffe (1930–31) noted a maximum rise in total blood sugar to only 115 mgm. after 30 to 50 grams of the ketose sugar. A level higher than 125 mgm. is considered abnormal.

Ordinarily, there does not seem to be an immediate effect of fructose on the glucose content of blood although injected fructose is sufficiently rapidly converted to glucose in the hepatectomized dog to prevent the onset of hypoglycemic convulsions (Bollman and Mann, 1931). Wierzuchowski, Pieskow, and Owsiany (1931), however, could not demonstrate any change in blood glucose in dogs receiving fructose intravenously at a constant rate of 2 grams per kilogram per hour over a 3 hour period although the blood fructose increased to a maximum of 198 mgm. per cent. Harding, Nicholson and Armstrong (1933) noted a

small elevation of blood glucose after the oral administration of fructose but not after mannose or xylose. Meyer (1926) attributed the hyperglycemia occurring 10 to 60 minutes after the oral administration of fructose to a glycogenolysis of liver glycogen caused by a reflex stimulation of the ketose sugar on the gastric mucosa.

Determination of galactose in blood. Galactose was determined by Raymond and Blanco (1928) by differential adsorption of glucose and galactose on baker's yeast. Corley (1927) differentiated glucose and galactose by determination of the reducing power before and after fermentation with yeast in which case the galactose concentration was considered equivalent to the non-fermentable portion. Similar technique was employed by Harding and van Nostrand (1929–30). In the later work Harding and Grant (1931–32) employed a "galac" yeast to determine the galactose by fermentation after removal of the sugars fermentable with ordinary yeast. Harding, Nicholson and Grant (1932–33) refined the technique by using *Saccharomyces marxianus*, which is somewhat more specific and without action on maltose. Harding and Grant (1932–33) have further revised their method so that glucose, galactose, and residual substances can be determined on 0.5 cc. of cutaneous blood. Another procedure for the determination of galactose and glucose in 0.2 cc. of blood is reported by Fishberg and Dolin (1930).

Factors altering the galactose concentration in the blood. Galactose is not present in the blood of normal men or women except for a short period immediately after its ingestion (Harding and Grant, 1931–32; Harding and van Nostrand, 1930; Harding and Grant, 1932–33). The last investigators demonstrated a galactemia in all cases 30 to 60 minutes after the ingestion of small quantities of galactose. After 40 grams of this sugar are ingested, the maximum height of blood galactose is reached during the first hour after which a rapid drop to zero usually obtains within two hours. The maximum quantity found in the blood under such conditions does not exceed 30 mgm. per cent. Although occasional increases to 60 mgm. per cent were found in normal men after the ingestion of 80 grams of this sugar, chiefly the duration of the galactemia is increased. As in the case of glucose the venous concentration of galactose was shown to be constantly lower than the arterial level which would indicate its withdrawal from the blood by the tissues.

The administration of galactose to dogs usually results in a much greater elevation in blood sugar than obtains after equivalent amounts of glucose or fructose. Wierzuchowski, Pieskow, and Owsiany (1931)

noted that after injections of the sugars at a constant rate into dogs, the galactemia reached a high level of 756 mgm. per cent.

The height of the galactose tolerance curve probably is not so readily influenced by fasting as is that of glucose. Although Reinhold and Karr (1927) did report somewhat higher blood sugar curves with galactose after 4 days of starvation from what was observed after 18 hours of fasting, the results of Goldblatt (1925), Corley (1928), Nitzulescu, Cumanov, and Cerkez (1932), Harding and Grant (1932–33), and MacKay, Bergman, and Barnes (1935) indicate that the increase in blood sugar is within normal limits after varying periods of fasting.

The level of galactemia after 40 grams of galactose is markedly lowered by the simultaneous ingestion of glucose, and the increased value for blood glucose may also be less than in control experiments. As noted in the earlier discussion this paradox is probably to be ascribed to an alteration in the absorption rate of the two sugars. According to the experiments of Corley (1927b) on rabbits and Cori and Cori (1927–28) on rats, cats, and rabbits, no change in blood glucose level follows the ingestion of galactose. Wierzuchowski, Pieskow and Owsiany (1931) did not find any rise in blood glucose after the administration of galactose at a constant rate of 2 grams per kilogram hourly to dogs by the intravenous route. In fact, the blood glucose fell due to the attendant blood dilution. Wierzuchowski (1931b) in later experiments in which the galactose injection was continued at the same rate as in the earlier tests for 10 hours noted no rise in blood glucose which was attributable to the galactose injected.

However, some data are found in the literature in which a concomitant rise in blood glucose followed the administration of galactose although the melituria which obtained consisted of a non-fermentable sugar (Blanco, 1928; Harding and van Nostrand, 1929–30; Bloch and Weisz, 1930; Harding and Grant, 1932–33). Bloch and Weisz (1930) believe that the initial rise in blood glucose after the oral administration of galactose is to be traced to a discharge of the latter sugar from the liver by reflex action. After the absorption of galactose into the blood, this mechanism is inhibited and the blood glucose reaches a subnormal level. Harding and Grant (1932–33) have demonstrated that the rise in blood glucose is not dependent on the amount of galactose ingested and that the hyperglycemia so obtained is quite transient. Because of the ephemeral nature of the rise in blood glucose, they do not consider it as evidence of a direct transformation of galactose to glucose. Neither is there proof of the existence of a reducing substance in the blood which is an intermediary of galactose.

Roe and Schwartzman (1932) found that an increased glucose occurred in the blood of some of their diabetic patients after galactose was administered. They believe this increase is traceable to its conversion to a fermentable sugar. In the case of normal rabbits, Roe and Cowgill (1935) demonstrated that the fall in blood galactose over a four hour period following this sugar sometimes occurred concomitantly with a gradual steady increase in blood glucose. Wierzuchowski (1931b) noted that the assimilation of galactose by dogs receiving a continuous injection of 2 grams of this sugar per kilo per hour over a 10 hour interval was not affected by the type of previous food, by hunger, by adrenalin or by thyroxin. Insulin alone diminished the proportion excreted in the urine. These results give further evidence that the factors important in affecting the removal of glucose from the blood stream are without influence on the assimilation of galactose.

COMPARATIVE GLYCOGEN FORMATION AFTER GALACTOSE AND FRUCTOSE. Probably the sugars are removed from the blood by the formation of glycogen before contributing to the blood glucose or possibly before undergoing oxidation. The ease with which fructose and galactose may be metabolized as compared with glucose is roughly in the same order as their glycogenic ability.

A summary of the literature seems to indicate that fructose is a better and galactose is a poorer glycogen-former than is glucose. Ishimori (1913) showed that glycogen deposition followed the intravenous injection of glucose or fructose in the rabbit but did not occur after galactose. Cori (1925–26a; 1926) noted that the rate of increase in liver glycogen after the administration of fructose to male rats was more rapid and reached a higher maximum than had previously been noted in similar tests on glucose (Cori, 1925–26b). Thus, following fructose the maximum liver glycogen which occurred after 5 hours averaged 4.23 per cent while the highest value obtained after glucose was 3.68 per cent on an average after 4 hours. The difference is not to be ascribed to variations in rate of absorption of the two monosaccharides inasmuch as Cori (1925) demonstrated that the glucose was taken up from the gastro-intestinal tract much more rapidly than was fructose. The per cent of the fructose absorbed which was deposited as glycogen reached a maximum of 39.4 per cent (18.2–39.4) in 4 hours, while the maximum efficiency of glucose was only 16.8 per cent during the same interval (16.8–6.1). Bertram (1929) also noted that fructose caused a better glycogen formation in the liver than glucose even in the absence of insulin.

The results on glycogen formation after galactose are quite uniform in indicating its weak glycogenic ability. Wieland's results on lactose (1899) were among the first to demonstrate this fact while the widely quoted tests of Murchhauser (1911) carried out on dogs evaluate galactose and lactose as only 13 and 7 per cent as effective as glucose. The latter investigator, however, made only single experiments on each sugar on animals of widely divergent size and breed. Peters and Van Slyke (1931) consider the failure of lactose to respond better may be related to its slow hydrolysis and absorption.

Only a minimum deposition of glycogen followed the administration of galactose to rats in the experiments of Cori (1926). The maximum value for liver glycogen noted was 1.36 per cent after 4 hours although 60 per cent of the absorbed galactose was being excreted during the fourth hour. On the basis of the amount of sugar *retained* rather than on that *fed*, galactose appears somewhat more satisfactory as a glycogenic agent. When compared by the latter method, the efficiency for galactose during the fourth hour was 12.3 per cent as compared with a value of 16.8 per cent for glucose during the same period. Cori (1926) states that "galactose plays an unimportant rôle as a source of liver glycogen." Somewhat more satisfactory results on glycogen formation after galactose were obtained by Deuel, MacKay, Jewel, Gulick and Grunewald (1933) on fasting dogs. When the liver glycogen was compared after equal amounts of the two sugars were retained, the level of liver glycogen 6 hours after glucose averaged 3.94 per cent compared with 3.36 per cent for a like period after galactose. These results are discussed by Cori and Cori (1934) who maintain that the procedure of comparing glycogenic ability of glucose and galactose in dogs on the basis of the amount retained is a questionable one. The liver is offered galactose in approximately twice the concentration as glucose. Moreover, because of the greater amount of galactose fed, the absorption requires a greater length of time than the smaller quantity of glucose does. The galactose is thus offered to the liver over a longer period than is glucose. Cori and Cori also believe that it is necessary to analyze the liver during the period while active absorption is in progress in view of the fact that the level of liver glycogen starts to fall promptly after the cessation of absorption.

Deuel et al. (1933) also point out that galactose in all probability serves as a much more satisfactory glycogenic agent in man than in rats and dogs. In the human subject only 4 per cent is excreted after a dose equivalent to 37.5 grams per square meter of body surface while

more than 50 per cent is excreted when a 30 per cent larger dose is administered to rats (47 grams per sq. m.). Cori (1933) and Harding, Grant and Glaister (1934) agree that species differences have to be considered in investigations concerning galactose in view of the fact that the rat and the dog have a particularly low tolerance for this sugar.

A possible method to avoid the obstacle encountered in experimental animals because of the galactosuria is in the employment of a species in which the excretion of this hexose is less complete. Emslie and Henry (1932) state that chickens excrete only small amounts of galactose although they absorb this sugar about as readily as glucose. These investigators noted glycogen formation in appreciable amounts only after glucose while galactose along with such well known glycogenic agents as glycerol, dihydroxyacetone and sodium pyruvate gave negative results. Bergman and MacKay (1935), however, have noted that the glycogen formation in nephrectomized rats reached a higher level 12 hours after glucose than after comparable amounts of galactose.

Glycogen retention after galactose and glucose. Although the glycogen deposition in fasting rats after galactose usually was much less than after comparable amounts of glucose, Deuel, MacKay, Jewel, Gulick and Grunewald (1933) have noted that the retention of glycogen was almost invariably higher in the galactose experiments. In tests on dogs the highest level of liver glycogen which was found 12 hours after the administration of galactose amounted to 5.10 per cent which exceeded the value for the glucose test at any interval. The comparative values for all the subsequent periods up to 72 hours continued higher than those of the glucose controls.

Even after the administration of single doses of galactose to fasting rats, it was demonstrated that the level of the glucose-fed animals had fallen to that of the galactose-fed ones after 24 hours which were still considerably higher than the fasting controls. The most striking differences were almost invariably noted in the glycogen of rats fasted for various periods after having previously been fed for a period of 7 to 12 days on a high galactose, lactose, or galactose-glucose diet when compared with those previously on a similar glucose-containing diet. In the former cases, the mean of both the liver and muscle glycogens was consistently higher for various periods up to 72 hours than the values for the glucose controls. The per cent of galactose absorbed, which was present in the liver of nephrectomized rats as glycogen in the experiments of MacKay, Bergman and Barnes (1935), reached a

level of 4.5 per cent as compared with a value of 3.1 per cent for glucose after 24 hours.

Such a variability could not be ascribed to a difference in the amount of liver glycogen at the start of the fast but may indicate that the glycogen originating from galactose is retained longer. These results are of special significance in relation to the greater ketolytic effect of galactose in rats and man which are discussed later.

Other polysaccharides formed from galactose. Although the glycogen formed after galactose has always been considered identical with that synthesized from glucose, the discrepancies in the level of glycogen after the two sugars might be ascribed to a difference in the end products of variable types of glycogen. Jewel (1932) found that glucose alone originated after the enzymatic or acid hydrolysis of the liver glycogens of dogs prepared after the administration of large amounts of glucose, fructose, or galactose. Not only were such hydrolysates completely fermentable with yeast but all gave an optical rotation of glucose. There was no evidence of other monosaccharide osazones than that of glucose which would seem to exclude galactose but not fructose as possible components of such glycogens. Harding, Grant, and Glaister (1934) were unable to obtain any other sugar than glucose from the liver or muscle glycogen of rats previously fed galactose.

Another possible method of explaining the discrepancies in glycogen retention after galactose and glucose might be that the different glycogens, although all composed of glucose as units, possess different linkages and consequently are decomposed with varying ease. One does not question the individuality of cellulose and glycogen although both of these polysaccharides are composed of glucose. As a possible confirmation of this idea are the experiments of Jewel (1932) who noted that the glycogen from galactose-fed animals was hydrolyzed somewhat more slowly than those samples from glucose or fructose-fed dogs. The variation in behavior of abalone glycogen from all of the liver glycogens was continually noted although it also yielded glucose.

A third possible explanation for the variations is the formation of other polysaccharides resembling glycogen after the administration of galactose. Such substances have recently been separated in quite pure form and shown to be distinct from glycogen. May (1931) isolated a substance which he called "sinistrum" from the body of the *Helix pomatia*. Sinistrum gave only galactose on hydrolysis; it had a much lower specific rotation than glycogen; finally, it was not colored by iodine solution. Subsequently, May (1931–32a) found the rotation

of this compound to be −22.7° in comparison with a value of +196.6° for glycogen. In distinction to glycogen, it was not precipitated by tannic acid. "Galactogen" as May now calls sinistrum, was found to account for 17.45 per cent of the polysaccharides obtained from the snail. Only galactogen was found by May (1931–32b) in the eggs of the *Helix pomatia* in which it makes up 37.8 per cent of the solid matter. On hydrolysis 109.4 per cent of galactose originated from the polysaccharide. The hydrolysis product was found to be beta-d-galactose with a specific rotation of +53.6°. In spite of the proximity of the specific rotation to that of glucose, its characterization as galactose was made certain by its quantitative determination as mucic acid. May and Kordowich (1932–33) found that glycogen and galactogen could not be differentiated by the Best method of staining *in situ* although the procedure was useful when interpreted in the light of the reaction with iodine.

Support to May's work is given by the experiments of Bierry, Rathery and Magnan (1932) who state that they have separated a second non-reducing polysaccharide in addition to glycogen from the liver of a dog. This yields galactose among other sugars on hydrolysis. It is called "glucidogene" by these investigators.

Further experimental evidence is necessary to prove whether the variations noted by Deuel et al. (1933) in glycogen retention after galactose are definitely to be ascribed to one or more of these causes. The results of Jewel (1932) seem to exclude "galactogen" as a constituent of his preparations. There is a possibility that such a polysaccharide might have been lost in the purification of the glycogen by the latter investigator while it was determined in the quantitative procedures employed by Deuel et al. (1933).

The evidence seems quite conclusive in indicating that fructose is somewhat superior to glucose in its ability to form glycogen. On the other hand certain species of animals such as the rat, the dog, and the rabbit possess only a limited tolerance for galactose and the glycogenic ability seems inferior to that of glucose. However, even in these animals values as high as 5 per cent have been reported for liver glycogen after galactose. Also, there is some evidence that the breakdown of liver glycogen after a galactose diet is somewhat less rapid than after glucose. Whether the polysaccharide, galactogen, plays an important rôle in the galactose metabolism of higher animals is undecided.

THE RELATION OF PHOSPHATES TO THE METABOLISM OF THE HEXOSES. Blood and urine phosphates were found to be lowered most by fructose,

followed in turn by glucose and galactose (Barrenscheen, Doleschall and Popper, 1924; Barrenscheen and Berger, 1924). On the other hand, Wierzuchowski, Pieskow and Owsiany (1931) found that the inorganic phosphate of the blood dropped most in dogs receiving galactose at constant rate intravenously while an intermediate effect was noted for fructose and the least with glucose. The maximum decrease in the blood phosphate noted in each case 3 hours after the start of the sugar injection was respectively 2.35, 2.04 and 1.60 mgm. Similarly, the level of urine phosphates reached the lowest values with galactose and the highest ones were noted with glucose. The compensatory rise after the cessation of sugar injection was in the reverse order as one might expect. If the lowering of blood and urine phosphate is proportional to the extent of the metabolism of the sugar, then it is evident that the highest metabolism is obtained after galactose.

THE OXIDATION OF FRUCTOSE AND GALACTOSE. *Effect on respiratory quotient.* That a variability in the rate of oxidation of fructose and galactose as compared with glucose follows the administration of these sugars is evident when one compares the respiratory quotients at various periods after the ingestion of the sugars. It is universally agreed that the rise in R.Q. occurs very much more promptly after fructose than after equivalent amounts of glucose. This was first noted by Tögel, Brezina and Durig (1913) and has been confirmed by Higgins (1916), Benedict and Carpenter (1918), Bornstein and Holm (1922), Holm (1923), Tsubura (1924), Lublin (1926), Deuel (1927), Cathcart and Markowitz (1927), Brechmann (1927), Campbell and Maltby (1928), Bertram (1929), Carpenter and Fox (1930a, b), Wierzuchowski and Boothby (1931), and Bachmann and Haldi (1935). Schirlitz (1927) failed to note any striking variation in the behavior of fructose and glucose on the R.Q. Carpenter and Lee (1932) found that when 20 grams each of glucose and galactose, glucose and fructose or galactose and fructose were ingested, the increase in the R.Q. was additive, i.e., equal to the sum of the net rise over the basal level obtained when each sugar was taken separately. After 40 grams of lactose the rise was somewhat less but more prolonged than after an equivalent amount of the hydrolysis products.

As an example of the comparative effect on the R.Q. (Deuel, 1927), the ingestion of 75 grams of sucrose or fructose was followed by a rise in quotient to a maximum level which generally exceeded unity within 10 to 20 minutes. The elevation in R. Q. after galactose was immediate and the highest values were usually obtained after 50 minutes in which

a value exceeding unity was found. In contrast to these results practically no effects were noted in the R.Q. for 45 minutes after the ingestion of comparable amounts of glucose or of maltose while the maximum value in R.Q. was not reached until the second or third hour and this never reached unity. For technical reasons the results of Lusk (1915) could not be obtained sufficiently soon to determine the R.Q. during the first hour after administration of the sugar. The averages of the second to the fourth hours noted by Lusk in his original experiments on dogs were 1.02 for fructose and sucrose, 1.00 for glucose, 0.93 for galactose and 0.90 for lactose.

Higgins (1916) as well as Cathcart and Markowitz (1927) attribute the higher R.Q. after fructose, sucrose and galactose to a greater rate of fat formation from the ingested carbohydrate rather than to a preferential combustion or to a quicker rate of absorption of the particular sugar.

The very rapid alteration in the R.Q. obtained within a few minutes after the ingestion of fructose has opened up the question as to whether it is indicative of a true metabolic process of combustion. An increasing amount of experimental data is available which indicates that the production of lactic acid occurs in sufficient quantities definitely to alter the acid-base balance and so to result in the blowing off of CO_2 from the blood and tissues. Abraham (1926a, b) demonstrated by *in vitro* experiments an increase in lactic acid after 4 to 6 hours when sterile defibrinated blood buffered at pH of 8.0 was mixed with various sugars. In this experiment the greatest acid production obtained with glucose and mannose, considerably less of an effect was found with fructose while galactose gave only a slight lactic acid production.

The extent of the acid-producing effects of the sugars have been reversed in most of the tests made *in vivo*. Katayama (1926) found an increase in the lactic acid content of human blood after the oral administration of glucose in doses of 1.75 gram per kilo. Similar results are reported by Mendel, Engel and Goldscheider (1925) and Wierzuchowski and Gamodska (1927a). On the other hand, Taistra (1921) could detect no change in the CO_2-combining power of the blood of a dog 2 to 4 hours after 50 grams of glucose orally although a marked drop resulted when 7.8 grams of glycollic acid or 8 grams of lactic acid were so administered. Rose, Giragossintz, and Kirstein (1930) found that the administration of 25 grams of fructose to a dog under amytal anesthesia was followed by a rise of 50 to 100 per cent in the lactic acid

content of the portal blood although no change occurred after glucose. Oppenheimer (1928) noted no increase in blood lactic acid in normal persons or those with diseases of the liver after the ingestion of 50 grams of either glucose or fructose. Lanyi (1926) found that the administration of glucose to dogs and men did not result in an increased blood lactic acid although the administration of fructose was followed by an increase in blood lactic acid during a 3 hour period. Similar differences between fructose and glucose were observed by Wierzuchowski and Laniewski (1931) in the blood of dogs which received a constant intravenous injection of the sugar at the rate of 2 grams per kilogram per hour for 3 hours. In the latter tests it was also demonstrated that the injection of galactose was unable to cause the production of a very great amount of lactic acid. The average maximum increases in milligrams per cent noted for each sugar during the injection period were as follows: fructose 10.5, glucose 8.6, and galactose 7.3. Orskov (1932) reports that the increase in lactic acid after fructose was 3-fold while smaller increases were noted after glucose and galactose.

More pronounced differences were noted in urine lactic acid excretion by Wierzuchowski and Laniewski (1931). This amounted to an average value in grams for an 8 hour period following the start of the sugar injection of 101.0 for glucose, 190.4 for galactose as compared with 545.8 for fructose. Moraczewski and Lindner (1921) also reported a much greater lactic acid excretion after the admistration of fructose intravenously to men than after glucose.

Extensive studies on the effect of tolerance tests with various sugars on the CO_2-combining power of the blood have been made on men by Campbell and Maltby (1928). These authors noted a considerable rise in lactic acid with a concomitant lowering in CO_2-combining power after fructose, sucrose, and dihydroxyacetone while the changes after glucose, maltose, lactose, and galactose were inappreciable. However, although the maximum lowering in CO_2-combining power after fructose was 10 volumes per cent, in one-half of the tests with fructose the decrease was less than 5 volumes per cent. The results from this standpoint hardly seem conclusive. It is unfortunate that simultaneous studies were not made on respiratory metabolism. Using the values of Palmer and Van Slyke (1917) that the retention of 1 gram of $NaHCO_3$ is equivalent in a 38 kilogram man to raising the CO_2-combining power of the blood 1 volume per cent, it is calculated that sufficient CO_2 is blown off by a 70 kilogram man when the CO_2-combining power falls

8 volumes per cent to account for an increase in basal R.Q. from 0.80 to one of 1.31. It should be noted that Holm (1923) was unable to demonstrate any alteration in the R.Q. of human subjects when 2 to 10 grams of sodium bicarbonate were administered.

Opposed to these views that the greater elevation in R.Q. after fructose is to be traced to an upset in acid-base balance are the results of Carpenter and Lee (1933b). Using their special method for simultaneous determination (1933a) of alveolar R.Q. and the R.Q. of the expired air, they conclude that the attendant acidosis is insufficient to account for the greater elevation in R.Q. after fructose. If the constancy in alveolar CO_2 indicates the true metabolic R.Q., then the net rise in R.Q. after fructose must indicate the metabolism of fructose without the formation of organic acids in sufficient concentration to affect the CO_2 in alveolar air. Carpenter and Lee believe that the interpretation of the earlier experiments in which an accumulation of lactic acid occurred after fructose, as merely a shift in acid-base balance rather than an oxidation, may be in error. They state the case as follows:

> Most of the studies reported in literature indicate that an increase in the lactic acid content of the body results from an introduction of fructose. However, many of the investigations were made by the intravenous injection of sugar solution and it is not improbable that the character of the metabolism of a substance differs according to the route by which it is introduced. In a number of studies the doses were large and over-loading may have resulted in abnormal metabolism. In some studies the ingestion of glucose also brought about similar changes in the blood reaction and lactic acid content although not always to the same degree as was brought about by the introduction of fructose.

The criticism of Carpenter and Lee that intravenous injection does not represent a physiological mechanism seems well founded from the recent work of Bollman and Mann (1931) in which it is shown that the intestines as well as the liver may be concerned in the transformation of fructose to glucose. The results of Carpenter and Lee are supported by those of Douglas and Priestley (1924) who found that the alveolar CO_2 remained practically unchanged after the ingestion of 80 grams of sucrose.

If the early rise in R.Q. after fructose was caused largely by a blowing off of CO_2 due to the accumulation of lactic acid in the blood and tissues, then an equivalent compensatory retention must occur in the later periods when the acid is being removed. A decrease in R.Q. below the basal level should obtain during the second phase of the metabolism

of fructose. The whole process should be concluded within 4 hours as Campbell and Maltby (1928) have shown that the adjustment of acid-base equilibrium is completed then after 100 grams of sugar have been taken. However, in the experiments of Deuel (1927) for example, not only was there an abrupt increase in R.Q. within 30 minutes after fructose to a value of 1.00 or more, as compared with a value of only slightly above the basal level after glucose, but the values for respiratory quotient were consistently higher in the fructose experiments from the second to fourth hour thereafter. Even if the early rise in R.Q. be considered as merely the expression of an alteration in acid-base balance, then the corrected R.Q. during the compensatory period in the second phase must give values considerably exceeding unity. This still would indicate a larger oxidation as well as a greater fat formation after fructose than after glucose.

In summary galactose is probably oxidized or converted to fat more readily in man than glucose. The rapidity with which the R.Q. is elevated after the former sugar is much greater than with glucose; in addition, there are no alterations in acid-base equilibrium after this sugar which do not occur after glucose. That the high R.Q. after sucrose and fructose are to be traced to their more rapid oxidation seems open to question although more experimental work is necessary in which simultaneous observations on R.Q., blood lactic acid, and CO_2-combining power are carried out.

Specific dynamic action.[1] In a majority of experiments reported in the literature the ingestion of fructose has been found to be followed by a greater increase in heat production than has the administration of an equal amount of glucose. The original experiments of Tögel, Brezina and Durig (1913) demonstrated that the increase in metabolism following a definite amount of glucose was 4.25 per cent while that of fructose was 6.25 per cent of the caloric value of the foodstuff. In the classical experiments of Lusk (1915), after 50 grams of fructose the increase in metabolism was equivalent to 37 per cent of the basal metabolism while after an equivalent amount of glucose, the value was only 30 per cent. The rise after sucrose is 34 per cent which is intermediary between fructose and glucose as one would expect because of its composition. Johansson, Billstrom, and Heijl (1904) had previously

[1] In most cases the specific dynamic action is reported as percentage of the caloric value of the ingested carbohydrate that the metabolism is increased. Lusk compares the specific dynamic action on the percentage of increase in basal metabolism.

shown that the increase in carbon dioxide output was twice as great after 50 grams of fructose as after an equivalent amount of glucose in man. However, Lusk (1915) calls attention to the fact that it would be erroneous to conclude that this indicated twice the specific dynamic effect in view of the fact that the carbon dioxide elimination in excess of a R.Q. of unity represents very little heat production.

Burger (1921) found the specific dynamic action of fructose to be greater than glucose. Schirlitz (1927) noted an average specific dynamic action of 9.7 per cent after the ingestion of 100 grams of fructose by man while the value obtained after 35 grams were fed to a fasting dog was 7.8 per cent of its caloric value. Progressively lower results were obtained on sucrose, maltose and glucose. The specific dynamic action noted after the last sugar amounted to only 2.8 per cent.

On the other hand, Benedict and Carpenter (1918) showed an identical increase in metabolism equivalent to 6 per cent of the caloric value of the assimilated carbohydrate after the oral administration of glucose and fructose. Deuel (1927) obtained a specific dynamic action of 8 per cent for glucose and 7 per cent for fructose. The differences between fructose and glucose also are not evident from the extended observations of Carpenter and Fox (1930a, b) on the metabolism following the ingestion of amounts of fructose and glucose varying from 5.05 to 104 grams by a trained subject. Increases in metabolism varying from 2 per cent with 21 grams of fructose to 12 per cent of the caloric value of the ingested sugar for 104 grams of this same carbohydrate were noted. After glucose, specific dynamic action amounted to 4 to 6 per cent following administration of 5 to 36 grams and equaled 10 per cent after 52 grams were administered.

Experiments on galactose are somewhat more limited in number. Lusk obtained a smaller effect after the administration of 50 grams of galactose to a dog than after a comparable amount of glucose. The increase in metabolism after galactose was only 22 per cent of the basal metabolism while after the latter sugar it amounted to 30 per cent of that value. The ingestion of a similar dose of lactose by a dog was followed by a rise in respiratory metabolism of only 3 per cent. Lusk considers the low results with lactose as due to its failure to be absorbed because of the absence of lactase in the gastro-intestinal tract of this animal. No record is available of the proportion of galactose which was retained by the dog. In the experiments of Deuel (1927) the specific dynamic action of galactose was equivalent to that of glucose, namely, 8 per cent while that of lactose amounted to 7 per cent. Wier-

zuchowski (1931) administered glucose, galactose and fructose intravenously to dogs at the rate of 2 grams per kilogram per hour over a period of three hours. The values for specific dynamic action obtained by Wierzuchowski (1931) are higher than those previously noted following the oral administration of the sugars. Taking into consideration the total amount of sugar retained, it was noted that the increase in metabolism was 17.3 per cent of the caloric value of the galactose assimilated, 13.8 per cent for fructose, and 9.7 per cent for glucose.

The effect of a simultaneous ingestion of two sugars on the metabolism of each other was studied by Carpenter and Lee (1932). The increase in metabolism when definite amounts of fructose and glucose were given together was shown to be additive; that is, the augmentation in metabolism was approximately equal to that obtained when the increases in metabolism of like amounts of each of the component sugars were taken in separate experiments. However, the rises in heat production after combining 20 grams each of galactose and fructose or of equivalent amounts of galactose and glucose were much greater than the sum of the increases noted when 20 grams of each sugar were given separately. The specific dynamic action after the combination of galactose and fructose amounted to 6.8 calories compared with an increase of 2.1 when the individual experiments on each sugar given separately were summated. After a combination of galactose and glucose was given, the increased heat production amounted to 8 calories compared with 1.5 calories when the separate experiments were added together. When 40 grams of lactose were taken, the increase in heat production amounted to 7.2 calories which compares with the results of the mixture of its hydrolytic products rather than the sum of the values obtained when each of the constituent sugars was ingested separately. These authors suggest that the aberrant effect of galactose when combined with other sugars may be explained because it is readily oxidized and less readily transformed to glycogen when taken with other sugars. The possibility that more galactose is changed to lactic acid in the presence of another sugar is also brought forward. Lastly, more unabsorbed galactose in the gut due to the retarding effect of other sugars on its absorption might account for its increased heat production.

However, the conclusions of Lusk (1929) would seem to indicate that the presence of unabsorbed materials in the gut are not able to invoke an increased heat production. If "Darmarbeit" is not the cause of increased heat production, then it would seem that the last suggestion

of Carpenter and Lee probably is not tenable. Further investigations to elucidate the cause of the unusual effect of galactose when administered with the other monosaccharides would be very much worth while.

Lusk (1928) considered that the cause of the specific dynamic action of carbohydrates was the presence of a plethora of metabolites in the tissues and blood. The greater effect of fructose than glucose was attributed to the increasing number of metabolities due to the presence of trioses which were intermediates in the fructose-glucose change. Lactic acid itself apparently did not cause the specific dynamic action by its acid properties. Deuel (1927) noted that there was no correlation between the periods of maximum R.Q. and the periods of greatest specific dynamic action. Bornstein and Holm (1922), Holm (1923) and Schirlitz (1927) found that neither the R.Q. nor the specific dynamic action paralleled the blood sugar level. Wierzuchowski (1931) does not consider that the height of the blood sugar is the cause of the specific dynamic action of these sugars. He points out that the greatest increase in metabolism is brought about by fructose which causes the least rise in blood sugar. If any relationship exists, it is an inverse one between the blood sugar level and the specific dynamic action. The more readily the particular sugar is assimilated by the tissues, the lower its concentration in the blood.

Although the weight of evidence seems to indicate that fructose and possibly galactose possess a greater specific dynamic action than does glucose, the necessity of a sufficient number of experiments so that the statistical method can be applied is particularly needed in this field before an exact evaluation of the relative values of the different carbohydrates can be made.

THE TRANSFORMATION OF FRUCTOSE INTO GLUCOSE. There is no doubt that fructose can readily be changed into glucose and that the reverse change is also possible. That the first reaction is possible was first demonstrated by Reilly, Nolan and Lusk (1898) and by Lusk (1898) on phlorhizinized dogs. Deuel and Chambers (1925) showed that the "extra sugar" originating in the urine of phlorhizinized dogs after 16 grams of fructose was largely glucose and that the rate of excretion was approximately the same as after a like amount of glucose.

That the rate of transformation is somewhat slow is indicated by the smaller efficiency of fructose in acting as an antidote for hypoglycemic shock. The conclusive experiments of Mann and Magath (1922) on dehepatized dogs demonstrated that only if the fructose is given some-

time before the hypoglycemic convulsions develop can it be effective. Later Bollman and Mann (1931) showed that if fructose was given in sufficiently large amounts before the onset of a low blood sugar level, hepatectomized dogs could be kept alive many hours without the necessity of glucose. In the experiments of insulin shock by Noble and Macleod (1923) with rabbits and of Herring, Irvine, and Macleod (1924) with mice, fructose was much less effective as an antidote than was glucose unless it was given soon after insulin. When fructose was administered simultaneously with insulin, Corley (1929) noted a protection against insulin shock without any pronounced effect on the rate of the removal of the sugar. Immediate protection would have been afforded in all cases if fructose as such could effectively replace glucose without being changed to the latter sugar.

Although the lactic acid content of the blood rises higher after the administration of fructose than after glucose, Bollman and Mann (1931) do not believe it is an intermediate in this change. The quantity of lactic acid in the blood is insignificant in comparison to the fructose metabolized; moreover, although fructose can be changed to glucose in the hepatectomized dog, lactic acid does not possess this ability.

Site of transformation of fructose. That the liver is one site for the transformation of fructose into glucose is indicated by the earlier results of Mann and Magath (1922) who reported that the ketose sugar was unable to counteract the hypoglycemic convulsions in hepatectomized dogs. One would interpret such data as proving that the liver is the *sole* site of the change of fructose into glucose. Isaac (1914) also demonstrated that a rapid decrease of fructose and a concomitant increase in glucose resulted when a surviving dog's liver was perfused with Ringer's solution containing the former sugar.

In spite of the importance of the liver in fructose metabolism, only slight variations in its assimilation follow severe liver damage. Lampson and Wing (1926) report an appreciable alteration in the fructose tolerance of dogs after chronic poisoning with CCl_4. The experiments of Corley (1929) on rabbits, however, demonstrate only a slight effect on the tolerance curve of fructose by such hepatotoxic agents as $CHCl_3$, P, and hydrazine.

That other tissues than the liver may also be important in bringing about a conversion of fructose into glucose is evident from the recent experiments of Bollman and Mann (1931). Blood glucose was maintained at a level sufficiently high to prevent hypoglycemic shock for many hours in hepatectomized dogs when fructose was the sole carbo-

hydrate injected; however, in view of the fact that the injection of fructose was valueless if a complete evisceration had also been performed or if the intestines as well as the liver had been removed, Bollman and Mann believe that the intestines are a second site for the change of fructose to glucose.

That the intestines are not indispensable if the liver is intact was shown by the normal fructose utilization in dogs from which the stomach and intestines were removed. The delayed effect of fructose in the hepatized animal may be caused by the time required for the secretion of the fructose into the lumen of the gut, its conversion to glucose there, followed by its reabsorption as such.

Burget, Moore and Lloyd (1932b) question the obvious deduction from the results of Bollman and Mann—namely, that fructose is transformed to glucose by the intestinal mucosa and absorbed as the latter hexose. These investigators were unable to demonstrate the formation of any glucose in closed loops of ileum of normal unanesthetized dogs or in living segments of rabbits kept in oxygenated Ringer's solution. Moreover, the blood samples removed from the mesenteric vein showed fructose in all cases while none was present in the blood from the heart. Although these results as well as others discussed earlier indicate that fructose may be absorbed as such and carried in the blood in unchanged form, they do not preclude the possibility that a partical change to glucose may take place within the mucosa cells in the process of absorption. However, the results of Steinberg (1927) are difficult to correlate with such a view. This investigator noted that fructose could only be slightly utilized as compared with glucose when perfused through an isolated living small intestine of the dog. If the ketose sugar were as rapidly transformed to glucose in the intestinal walls as the experiments of Bollman and Mann (1931) indicate, then the glucose so formed should have been removed from the perfusion fluid in Steinberg's experiments. The positive results in the experiments of Bollman and Mann might be because intact animals rather than isolated organs were employed. Possibly the intestine is able to bring about the transformation to glucose only when it acts in conjunction with some other organ. Such a hypothesis would bring the conflicting results of Bollman and Mann (1931) and those of Steinberg (1927) and of Burget, Lloyd and Moore (1932b) into harmony.

Bollman and Mann (1931) believe the site of transformation of fructose to glucose is confined to the liver and intestines. In the absence of these organs it cannot be converted to muscle glycogen although such a synthesis occurred when the liver or intestine was intact.

DIRECT UTILIZATION OF FRUCTOSE. There is some evidence that fructose may be utilized without preliminary transformation to glucose or glycogen. Steinberg (1927) studying various isolated surviving organs demonstrated that the kidneys and skeletal muscles were able to utilize fructose directly as well as glucose while the liver and lungs used it better. The pancreas and the heart were completely unable to metabolize the ketose sugar. McGuigan (1908) and Griesbach (1929) also noted that the surviving muscle was able to utilize fructose as readily as glucose. Burger (1921) believes that fructose can be oxidized without conversion to glycogen. If the more rapid rise in R.Q. which follows the ingestion of fructose as compared with that of glucose is definitely proved to be traceable to a more rapid utilization of the former sugar, one has additional evidence that oxidation of the ketose sugar may occur without preliminary transformation to glucose. Otherwise, why should its metabolism be more rapid? On the other hand, if all monosaccharides (including glucose) must be transformed to glycogen before oxidation occurs, the readier utilization of fructose but not that of galactose might be so explained.

THE TRANSFORMATION OF GALACTOSE INTO GLUCOSE. There seems to be some evidence that galactose also can be converted into glucose. Although some of the experimental data fails to demonstrate an immediate effect of galactose on blood glucose, Roe and Schwartzman (1932) and Roe and Cowgill (1935) have noted an increase in blood glucose in diabetic patients and dogs respectively after galactose. The fact that galactose gives slight relief to the hypoglycemic convulsions during insulin intoxication as demonstrated by Noble and Macleod (1923) is also evidence of its convertibility to glucose. Data on the rapidity of such a conversion should be available from experiments on diabetic animals. Roe and Cahoon (1933), however, failed to note an appreciable increase in glucose excretion by depancreatized dogs after galactose was fed although all of the administered galactose was not excreted as such in the urine. Kosterlich and Wedler (1933b), on the other hand, did report an increase in glucose as well as galactose after the latter sugar was given to pancreatectomized dogs. Similar results were found by Bollman and Mann (1934) who reported that four-fifths of the 50 gram dose of galactose administered was excreted as "extra glucose."

Site of transformation of galactose to glucose. The liver apparently is the chief site for the conversion of galactose into glucose. Although Mann and Magath (1922) reported in their earliest work that galac-

tose possessed a slight therapeutic effect after hepatectomy, Bollman, Mann and Power (1935) more recently have been unable to prevent the onset of hypoglycemic convulsions in the dehepatized animals with relatively large amounts of this sugar. Based on this assumption, a number of liver function tests have been devised which attempt to determine the condition of the liver by the hyperglycemia or galactosuria arising from certain prescribed doses of this sugar. Shay, Schloss, and Bell (1931) conclude that it is an ideal sugar for liver function tests because it is easily absorbed from the intestine and readily excreted by the kidneys even with renal damage.

DIRECT UTILIZATION OF GALACTOSE. Although the liver is undoubtedly necessary for the conversion of galactose to glucose, the dog is able to metabolize considerable amounts of this sugar in the absence of the liver. Bollman, Mann and Power (1935) noted that the blood galactose curve was only slightly prolonged in hepatectomized dogs as compared with normal ones after the intravenous administration of 500 mgm. of this sugar per kilogram. On the other hand, the excretion of galactose in the urine was increased from about 20 per cent of the total injected to 50 or 60 per cent after hepatectomy. When hepatectomy and nephrectomy were both performed, the galactose entirely disappeared from the blood within four or five hours. Little effect was noted on the attendant galactosuria from experimental cirrhosis (produced by CCl_4, $CHCl_3$, P, or toluylenediamine) or from the removal of 50 to 70 per cent of the liver. Although McGuigan (1908) reported that the muscles were able to abstract galactose from the perfusion fluid, contrary results were found by Griesbach (1929) as by Roe and Cowgill (1935). The last investigators also demonstrated that the brain was unable to bring about a removal of galactose from the blood.

Although it has been generally supposed that galactose must be converted to glycogen or glucose before it can be metabolized, there are certain data which make such a hypothesis questionable. Bollman, Mann and Power (1935) consider the possibility that it is oxidized before being changed to glycogen or glucose although the animals were unable to survive long enough without glucose in the absence of the liver to prove absolutely that a muscle glycogen was not formed from it. They did demonstrate, however, that even after repeated administration of this sugar, the rate of disappearance from the blood was unaltered and that no storage of galactose as such occurred.

Moreover, the more rapid rise in the R.Q. noted after galactose than after glucose can only be interpreted on the basis of a more rapid oxi-

dation or conversion to fat. If a conversion to glycogen or glucose must precede the oxidation of galactose, why should a more rapid oxidation obtain? Certainly even though it is somewhat more rapidly absorbed than is glucose, its more sluggish conversion to glycogen or glucose should result in its lower concentration in the tissues than after the ingestion of a comparable quantity of glucose. In the molecular rearrangement which occurs in its transformation to glucose, lactic acid is probably not an intermediate.

On the other hand, Harding, Grant and Glaister (1934) believe that galactose must be converted to glycogen before it can be utilized. They base this hypothesis on the fact that no increase in liver or tissue glucose follows the oral administration of galactose in fasting rats although the liver galactose increased from 0 to 114 mgm. per cent and the muscle galactose from 0 to 33 mgm. per cent one hour after the sugar was given. No evidence was obtained that any other reducing carbohydrate was formed from galactose. The failure of a storage of large amounts of galactose in the liver after the oral administration of this sugar is interpreted by Kosterlitz and Wedler (1933c) to mean that galactose does not pass through an intermediary stage of glucose in the liver but that it passes directly to glycogen there. Roe, Gilman and Cowgill (1934–35) believe that galactose must be converted to glucose before it can be metabolized in the normal or depancreatized dog as well as in the human. The slower effect of galactose in raising the R.Q. in dogs than in men is interpreted as evidence of the retarded rate of this change in the dog; this obtains not because of a fundamental dissimilarity in cellular metabolism but because of a species difference in man due to an adaptation as a result of a greater galactose content in the natural diet. It should be noted, however, that Deuel, MacKay, Jewel, Gulick and Grunewald (1933) were unable to increase the galactose tolerance of rats by a prolonged high-galactose diet.

THE RÔLE OF INSULIN IN THE METABOLISM OF FRUCTOSE AND GALACTOSE. Fructose and galactose as well as other reducing carbohydrates act as stimulants for insulin secretion according to Grafe and Meythaler (1928) in a like manner to glucose. These results confirm the earlier ones of Reinhold and Karr (1927) that fructose and galactose are able to stimulate the glucose-removing mechanism in rabbits previously fasted, in a similar manner to what glucose acts. Fructose, however, is only about one-half as effective as glucose while galactose is somewhat less potent. Sawada (1933) also noted that galactose was less efficient in stimulating insulin secretion in the rabbit than were

glucose or fructose. More recently, MacKay, Bergman, and Barnes (1935) report that the administration of galactose to fasting rabbits does not alter the subsequent blood sugar curves following glucose or galactose. It is of importance to determine whether the pancreatic hormone is essential for the complete assimilation of such carbohydrates and if so, what particular reactions are regulated by it.

Effect of insulin on transformation to glucose. Isaac (1914) found that the transformation of fructose into glucose could be brought about by liver perfusion. Moreover, Bollman and Mann (1931) noted that the injected fructose was changed to glucose in hepatectomized dogs from which the pancreas had been extirpated. The speed of transformation of fructose does not seem to be altered when an excess of insulin is present. Although Bollman, Mann and Power (1935) could not demonstrate a change in galactose to glucose in the absence of the liver, such a transformation was noted by Bollman and Mann (1934) in depancreatized dogs with intact livers. Apparently the pancreas is not essential for the conversion of galactose or fructose into glucose.

Effect of insulin on sugar tolerance. If the pancreatic hormone is without influence on the transformation of the hexoses to glucose, and if the hexoses are only metabolized after conversion to glucose, insulin should have an identical effect on their metabolism as on that of an equivalent amount of glucose. Such, however, is not the case. When fructose and glucose were injected intravenously by Wierzuchowski (1926) into dogs at the constant rate of 2 grams per kilo per hour, it was noted that the attendant melituria was lowered by insulin in the glucose tests but no change in the sugar excretion occurred in the animals receiving fructose. The author attributes the fall in blood sugar in the experiments with fructose to a drop in the glucose preëxisting in the blood. Cori and Cori (1925-26; 1927a) noted no change in tolerance for fructose after insulin. Basch and Pollak (1927) also observed that the assimilation limit was increased for glucose when insulin was injected while that for fructose was slightly lowered and that for galactose largely decreased.

Wierzuchowski, Pieskow and Owsiany (1931) later confirmed the earlier observation of Wierzuchowski (1926) that insulin is without effect on the fructose elimination in dogs receiving this sugar at a constant rate intravenously. However, they noted that the tolerance for intravenous galactose is markedly increased by insulin. The amount of galactose retained was increased about 40 per cent by the administration of insulin (30.4 to 42 per cent) while the proportion excreted

in the urine was reduced. In more recent experiments on 8 dogs, Wierzuchowski (1931b) found that insulin was the sole hormone which influenced galactose assimilation. The amount of this sugar excreted was reduced from 72.3 to 62.8 per cent by insulin while thyroxin and adrenalin did not alter the control level of galactosuria. Roe and Schwartzman (1932) believe that insulin does not affect the anabolism of galactose.

Effect of insulin on glycogen formation. Although the tolerance of intravenously injected fructose seems to be independent of insulin, Cori and Cori (1927b) consider that this hormone plays an active rôle in the fate of that orally administered. The amount of glycogen deposited in the liver is suppressed to a negligible value when insulin is given to fructose-fed rats; the effect of the pancreatic hormone on glucose-fed rats is similar but less intense (Cori, 1926).

Minkowski (1893) first demonstrated that glycogen formation readily occurred in depancreatized dogs after fructose was fed but not after glucose. Kosterlitz (1933) confirmed his findings. The latter investigator attributes the negative results of Cruickshank (1913) with fructose to the fact that the dogs were killed too long after the sugar was fed. Kosterlitz concludes that fructose is directly convertible to glycogen without the intermediary conversion to glucose, and that insulin is not necessary for the synthesis.

Effect of insulin on oxidation. The amount of fructose oxidized is largely increased in rats by the injection of insulin (Cori and Cori, 1927b). The ratio of glycogen formed to fructose oxidized in normal rats fasted 24 hours was decreased from 1.16 to 0.30 by insulin. These investigators were able to account for 81 and 90 per cent of the administered fructose in the two groups of animals in the above tests. The results were confirmed and extended in later work (Cori and Cori, 1928). Similarly, the increased tolerance for galactose noted after insulin has been associated with an increased oxidation of it (Wierzuchowski, 1931a).

Effect of insulin on lactic acid formation. The curves for blood lactic acid were found to be increased over those in the non-insulin tests by Wierzuchowski and Laniewski (1931) when this hormone was injected into dogs receiving a constant amount of glucose, galactose, or fructose intravenously. A larger proportion of lactic acid also was excreted in the urine. The average total urine lactic acid (in milligrams) for the period investigated during and after the sugar administration without (1st value) and with added insulin (2nd value) were the following:

glucose 101.0, 198.0; fructose 545.8, 741.8; and galactose 190.3, 303.4. Thus, the relative position of these sugars in producing lactic acid is not changed by insulin.

Effect of insulin on blood and urine phosphate. The injection of insulin lowers the blood and urine phosphate as noted by Harrop and Benedict (1922–23), Wigglesworth, Woodrow, Smith and Winter (1923), Perlzweig, Latham and Keefer (1923–24) and others. Wierzuchowski, Pieskow, and Owsiany (1931) found that the level of inorganic phosphate in the blood was decreased most after insulin in the dogs receiving a continuous injection of galactose, while the decrease was slightly less in turn after fructose and glucose. The maximum decreases after insulin were 2.72, 2.62, and 2.55 mgm. with the three sugars respectively. Not only was the drop in blood phosphates more precipitous in all cases after insulin but the low level persisted for at least 5 hours in distinction to the much shorter period in the control experiments in which the sugars were given without the hormone. Moreover, the extent of the drop in blood phosphate was augmented by insulin. Urine phosphate values were also lowered practically to blank values with all the sugars for as long as 8 hours after the insulin. The increased metabolism of fructose and galactose caused by insulin brings about a similar although a slightly greater phosphate removal than does glucose.

Utilization of hexoses in diabetes. The search for carbohydrates which can be metabolized by diabetics without the necessity of insulin has involved a consideration of the possibility of using fructose or galactose. Folin and Berglund (1922) have suggested that the latter sugars might be so employed advantageously.

Fructose as such or in the form of the polysaccharide, inulin, has been shown to bring about an increase in R.Q. of diabetic patients by Root and Baker (1925) while Carpenter and Root (1928) showed that an absorption and utilization of the carbohydrates of Jerusalem artichoke occurred in diabetics without an attendant glycosuria. Similar results are reported by Joslin (1928) in which 100 grams of inulin provoked no glycosuria while a control experiment with a similar amount of potato starch resulted in an excretion of sugar. Although Carpenter and Root did demonstrate the disappearance of a considerable amount of inulin from the gastro-intestinal tract, the current opinion has been that inulase is not present in the digestive secretions of the human subject. Lusk (1928) was of the opinion that fructose could not be oxidized by the complete diabetic while von Noorden and Naunyn

(1928) believe that any superiority of fructose disappears when continuously administered.

The difficulties in making adequate controls on diabetic patients are such that other criteria are desirable. Campbell and Markowitz (1927) could demonstrate no differences in the utilization of glucose, fructose and inulin by depancreatized dogs. Kosterlitz (1933) reported that depancreatized dogs store considerable amounts of glycogen after fructose while glucose feeding results in an inappreciable storage of this polysaccharide. The urinary glucose was found to be significantly less after the administration of four 40-gram doses of fructose to pancreatectomized dogs than in other similar dogs which received glucose. Abe (1934) also noted that partially diabetic dogs utilized fructose better than glucose. Although Bollman and Mann (1934) reported that fructose or sucrose (in distinction to glucose) was partially utilized for short periods when fed to pancreatectomized dogs, their results indicate that the superior effect gradually became less pronounced and most of the administered fructose appeared in the urine as glucose. The differences during the early periods between these sugars are particularly striking. After glucose was administered, the glucose elimination in one experiment gradually increased from 45.1 grams on the first day to 68.2 grams on the fifth day with a mean daily excretion of 54.7 grams for the test. A subsequent trial of fructose on the same dog gave a glucose excretion of 16.8 grams on the first test day which gradually rose to 19.8 grams on the fifth one. The daily average was 17.8 grams. In a second protocol they report an average urine sugar after glucose of 71.1 for 5 days, while the results on 3 successive tests of like duration with fructose gave averages of 25.2, 56.2 and 63.6 grams respectively. However, the tolerance for glucose was not determined after the fructose tests. It might have resulted in much larger glycosuria than 71.1 grams. Similar results were obtained on 4 other dogs although the protocols are not reported. The urine nitrogen was also lower after fructose indicating a greater sparing action on protein metabolism by the ketose sugar. Fructose did not remain unabsorbed in the gastro-intestinal tract nor was it excreted as such. The ability to utilize large amounts of fructose after continued feeding of it was regained after its elimination from the diet for a month. Inulin was only slightly hydrolyzed in the intestines of the depancreatized dogs, but those portions which were hydrolyzed met the same fate as the ingested fructose. Although Bollman and Mann intimate that fructose is of little value in diabetes because of the short period

during which it exerts a beneficial effect, it would seem to the reviewer that this variability in fructose assimilation is sufficiently marked to make a further study of its applicability in diabetes of considerable value.

As a possible explanation of the gradual lowering of the utilization of fructose after its continued feeding to diabetic dogs, the following hypothesis may be proposed. When fructose or sucrose is absent from the diet for a long period, the efficiency of the transformation of fructose to glucose becomes much less. Fructose which is then administered is only slowly converted to glucose and largely follows its own pathway of metabolism (glycogen and fat formation, oxidation). After repeated doses of the ketose sugar, the ease of transformation to glucose increases until it is rapidly converted to the latter sugar. When such a condition obtains, fructose follows the course of metabolism of glucose; since the latter sugar cannot be utilized by depancreatized dogs, the assimilation of fructose under such conditions is no better than that of a like quantity of glucose. When it is removed from the diet, the facility of its transformation to glucose is gradually lessened and a superior utilization of fructose over glucose again obtains. In support of such a theory it is well known that certain types of foodstuffs when continuously fed provoke the formation of digestive secretions high in the appropriate enzymes. Although we have no information of any ferment either in the intestine or liver which catalyzes the change of fructose to glucose, it seems that whatever mechanism is involved might also be gradually improved with continued stimulation to activity.

Galactose also has been reported to be more readily utilized by diabetics than glucose. Roe and Schwartzman (1932) noted that the galactose tolerance of diabetics was similar to that of normal individuals while the increase in total blood sugar in 7 of the 10 cases was no greater than in the normal subjects. Kosterlitz and Wedler (1933a) also have noted that the melituria and glycemia were less when glactose was administered to diabetics than when an equivalent amount of glucose was fed. On the other hand, Karr and Letonoff (1933) failed to note any greater oxidation by diabetics of orally administered galactose than of the other ingested carbohydrates.

The superiority of galactose over glucose is not so evident from experiments on diabetic dogs. Roe and Cahoon (1933) did find that approximately 50 per cent of the galactose fed to depancreatized dogs was utilized and no increase in glucose excretion was noted; some of their depancreatized dogs receiving galactose survived without insulin

for the longest periods on record. On the other hand, Kosterlitz and Wedler (1933b) noted that assimilated galactose was quantitatively changed to glucose in depancreatized dogs. Also, no difference was found in the utilization of galactose and glucose in partially depancreatized dogs which also received insulin. Bollman and Mann (1934) could find no variation in the total sugar excreted by depancreatized dogs after glucose or galactose although some of the later sugar was excreted unchanged. Roe, Gilman and Cowgill (1934–35) were unable to note any increase in the R.Q. of fasting pancreatectomized dogs when as high as 5 grams of this sugar were given orally; with normal dogs a rise in R.Q. occurred when 5 grams per kilogram were administered but not with a 2 gram dose. Because of the delayed rise in R.Q. in both instances these authors conclude that galactose can only be oxidized after conversion to glucose in the normal or diabetic dog. Roe and Cowgill (1935) in later work report further evidence that in adult dogs galactose is oxidized only after conversion to glucose.

The data on the superior utilization of fructose and galactose in diabetes mellitus are too extensive to be disregarded. The tests on such diabetics need to be rigidly controlled as far as the metabolism of glucose is concerned; blood and urine sugars should be correlated with the determination of respiratory metabolism; and finally, they need to be sufficiently extensive to rule out the biological variability. In drawing deductions from experiments on dogs and rats, one must remember the much smaller tolerance for galactose of these animals as compared with man. Because of this fact, the failure of galactose to show a superior action to glucose cannot be considered as proof that identical relationships hold in man. It is entirely possible that the small amount of insulin supplied by the diseased pancreas is sufficient to bring about a metabolism of fructose and galactose but not of glucose. Such a hypothesis would explain the superiority of the former sugars in diabetes mellitus and their failure to exhibit this property in completely pancreatectomized animals. Comparisons on animals possessing only a small remnant of pancreas might demonstrate similar variations to what has been noted on man.

KETOLYTIC ACTION OF FRUCTOSE AND GALACTOSE. Some light on the intermediary metabolism of the sugars may be obtained by a study of their ability to prevent ketosis. If galactose and fructose are completely changed into glucose, then their ability to prevent ketosis should be identical with that of an equivalent amount of glucose. Shaffer (1922–23) has made such an assumption in calculating the ketolytic action

of various absorbable carbohydrates. Goldblatt (1925) was the first to attempt a comparison in ketolytic effect of the various carbohydrates. When 50 grams of galactose, mannose, lactose, or glycerol were fed to normal men who had fasted 40 hours, no ketolytic action was observed while after an identical amount of glucose, fructose, sucrose, or maltose a marked drop in ketonuria occurred. This author believes that the former group of substances are not ketolytic agents. These experiments may be criticized in view of the fact that ketonuria obtained after a short period of fasting is only a moderate one and that the determination of ketolytic effect is based on a qualitative rather than a quantitative difference in acetone body output.

Deuel and Chambers (1925) had previously observed that the administration of fructose and galactose to a phlorhizinized dog was followed by a drop in nitrogen excretion similar to what was observed after glucose. Inasmuch as Wierzuchowski (1926) later noted that the nitrogen-sparing action following sugar ingestion in phlorhizinized dogs ran parallel with the decrease in acetone body output, it may be concluded from the experiments of Deuel and Chambers (1925) that fructose and galactose have a ketolytic effect in phlorhizinized dogs. A further comparison of the ketolytic effect of these sugars was made on normal subjects who were fasted or partook of a protein-fat diet by Deuel, Gulick and Butts (1932). The latter authors observed that the administration of galactose was followed by a greater drop in acetone body excretion as well as a more prolonged lowering than was caused by comparable amounts of glucose. Moreover, the minimum level of acetone body excretion usually was reached after a much longer period following the ingestion of galactose than was noted in the case of glucose. Whereas the acetone-body output in a subject on a constant protein-fat diet fell from an average of about 6 grams daily to 3.20 grams on the day when a single dose of 75 grams of glucose was taken and rose to 4.09 grams on the second day thereafter, the acetonuria after the same quantity of galactose dropped from 7.63 grams to 2.77 grams on the first day and reached a minimum of 2.32 grams on the second day thereafter. A return to the original level of ketonuria did not occur until the fourth day after the galactose. The results on fructose apparently demonstrated that it occupies an intermediate position between galactose and glucose as a ketolytic agent.

Butts (1934) noted that the ketonuria which developed in rats when sodium acetoacetate was administered immediately following a diet high in galactose was less than occurred in those after a comparable diet

containing glucose. The same level of ketonuria ordinarily was not obtained even by the third day of fasting and sodium acetoacetate feeding. The results on animals previously on a lactose diet were intermediate. The ketolytic action of galactose has also been noted in children by Bischoff and Heistermann (1930) and in diabetic subjects by Kosterlitz and Wedler (1933a).

In an attempt to explain this effect of galactose, Deuel, MacKay, Jewel, Gulick and Grunewald (1933) noted that the liver and muscle glycogen of rats and dogs dropped more slowly in those animals previously fed on a galactose diet than those which had received a glucose one. Inasmuch as Shapiro (1935) has demonstrated that only those intermediates of glucose which are glycogen formers are able to act ketolytically, one must assume that fructose and galactose exerted the ketolytic effect only after being transformed to glycogen or glucose. If one accepts the fact that ketosis can only be combated by the oxidation of glucose, then the superior ketolytic action of galactose and fructose can be explained on the larger percentage of sugar retained as glycogen, by the formation of a less readily hydrolyzed glycogen after galactose than after glucose or finally because of the formation of galatogen. Whether any of these possibilities are responsible for such differences must be decided after further investigations.

THE EXCRETION OF FRUCTOSE AND GALACTOSE IN THE URINE. The presence of a fructosuria or galactosuria after the oral administration or of the intravenous injection of the respective sugars has often been demonstrated. Deuel and Chambers (1925) noted the presence of a sugar possessing similar properties to fructose, i.e., destruction when boiled with 10 per cent HCl for 30 minutes, in the urine of phlorhizinized dogs following the administration of fructose by mouth. Cori and Cori (1927a) reported that while non-fasting rats tolerated 2.50 grams of glucose per kilo per hour intravenously by the femoral vein, only 0.35 gram of fructose could be administered without a fructosuria; in the mesenteric vein the dose of the latter sugar tolerated was 0.70 gram. Woodyatt, Sansum, and Wilder (1915) found that the tolerance of rabbits, dogs and men for constantly injected intravenous glucose approximated 0.85 gram per kilo per hour while they could only tolerate 0.15 gram of fructose and less than 0.10 gram of galactose. As discussed earlier the fructosuria under such conditions is not appreciably altered by insulin.

Folin and Berglund (1922) postulated that fructose possesses a renal threshold. Their demonstration of a positive Seliwanoff test in blood

plasma during absorption of fructose while the urine gave only a doubtful reaction convinced them that no fructosuria occurred when some fructosemia existed. Bodansky (1923), as a result of experiments on dogs, reached a similar conclusion and suggested that this ketose sugar has a renal threshold comparable with glucose. Spence and Brett (1921) do not consider fructose as satisfactory for liver function tests because of the renal threshold involved.

However, by somewhat more delicate methods Tashiro and Tietz (1930) using a modified Pettenkofer test showed that a positive-reacting substance (presumably fructose) was present in the urine of normal non-fasting subjects and the intensity of the reaction was increased after fructose or sucrose was given. Although blood from fasting subjects gave negative results with their reagent, a positive test followed the oral administration of sucrose. Harding and Selby (1931) noted the excretion of a small amount of fermentable sugar after fructose or fruits. Although Roe (1934) was unable to detect any fructose in the urine of fasting subjects, he demonstrated its presence after sucrose was ingested or fruits were eaten. The presence of definite quantities of fructose in the urine after moderate amounts are taken when the quantity in the blood rarely exceeded 10 mgm. per cent must indicate that the renal threshold for this ketose sugar is extremely low if one exists.

Bauer (1906), Hirose (1912), Wagner (1914) and Corley (1927a) have obtained a positive mucic acid test for galactose in the urine after the administration of this sugar. Bodansky (1923) was unable to obtain such tests on the urine of dogs after rather large doses of galactose. However, the other evidence supports the fact that the sugar in question is galactose. Reiss and Jehn (1912) separated galactosazone. Rowe and Chandler (1924) confirmed the latter work and also noted that the sugar gave a positive Tollen's test. Finally, Halberkann and Kahler (1926) were able to isolate galactose in crystalline form from the urine of patients previously given galactose.

No galactose is present in normal urine (Harding and Grant, 1931–32). After galactose is administered, there is evidence for the excretion of no other reducing metabolite (Harding and van Nostrand, 1929–30; Harding and Grant, 1932–33). When as small an amount as 10 grams of galactose is taken orally, Folin and Berglund (1922) noted a small excretion. In normal men the tolerance lies in the neighborhood of 30 to 40 grams. Although the galactose tolerance test was originally used as a criterion of liver efficiency, Harding and Moberly (1930)

indicate that it has little or no specific value. Rowe (1928) (1929) and Lawrence and Rowe (1929) are of the opinion that the galactose tolerance tests are of value in the diagnosis of certain endocrine disorders. Rowe reported that normal women possess a greater tolerance than men. Normal men showed a melituria on an average after 30 grams of galactose while 40 grams of the sugar were required before the sugar appeared in the urine with women. He also indicated that galactose tolerance was lowest during the prepubertal years (20 grams), while the highest values were obtained on the adult women during the intermenstrual period (40 grams). A lower tolerance was noted after the menopause (30 grams) and variations were noted during the menstrual period and pregnancy. Harding and Moberly (1930) using a quantitative determination of non-fermentable reducing substances (rather than a qualitative Benedict test as used by Rowe) could demonstrate no sex variability in galactose tolerance. Harding and Moberly attribute Rowe's results to a difference in concentration of the galactose in the urine due to a greater diuresis in the case of women. Roe and Schwartzman (1932) also were unable to note a sexual difference in tolerance to galactose.

In spite of a melituria after small amounts of galactose, it is a relatively well utilized sugar. Harding and Moberly noted an average excretion of only 450 mgm. after 30 grams were given orally to women. Harding and van Nostrand (1929–30) found an average of 2.06 grams after 50 grams of galactose (0.18–6.82) in 14 normal subjects. After 80 grams of galactose Harding and Grant report an average galactosuria of 2.65 grams.

Rowe and Chandler (1924) maintained that a renal threshold exists for galactose. However, because of the low concentration of galactose in the blood at which a galactosuria has been noted, it is now generally considered that a renal threshold for this sugar is non-existent. Folin and Berglund (1922) base their contention of this hypothesis on the ability of very small doses of galactose to produce extra urinary reduction substances. Goldblatt (1925), Harding and van Nostrand (1929–30) and Shay, Schloss and Bell (1931) arrive at a similar conclusion as a result of the demonstration of a rise in non-fermentable reducing substances in the urine without an appreciable increase in venous blood sugar. Harding and Grant (1932–33) state that if any threshold exists for galactose, it must be extremely low.

Toxicity of galactose. Guha (1931) reported that when rats and mice were fed on a fat-free diet containing large amounts of galactose

as the sole carbohydrate, death ensued following the development of beriberi-like symptoms. This investigator believes that death results from the inability to supply sufficient energy to the rat. In some cases death occurred within several days after high galactose diets. Such results, however, were not noted in a single case by Deuel, MacKay, Jewel, Gulick, and Grunewald (1933) in experiments on adult rats carried on for as long as 12 days in which galactose made up to 44 per cent of the diet nor in tests which extended over three months on rapidly growing rats in which galactose made up 20 per cent of the ingested food. Such a toxic effect also is not reported by Mitchell (1934–35). The results of Guha, of course, may possibly be traced to the very high level of galactose (70 per cent) employed in his diets.

Another remarkable effect of galactose has been reported by Mitchell (1934–35) who describes cataract formation in rats fed on diets containing 35 per cent of the sugar. Similar results were previously noted by Mitchell and Dodge (1935) in rats receiving a diet composed of 70 per cent of lactose. Mitchell attributes the deleterious effect of galactose to an action on the lens epithelium. Kirby, Estey and Weiner (1932) demonstrated that galactose is much more toxic on tissue cultures of this lens than is fructose or glucose. Mitchell believes that her results must prove the presence of galactose as such in the blood of her rats.

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ELECTRICAL PHENOMENA IN LARGE PLANT CELLS

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Studies of vital processes ordinarily suffer from variables created during the process of measurement and it is highly desirable to avoid such complications. Hence the importance of electrical measurements which permit us to study the cell with little or no disturbance of normal functions.

Such measurements allow us to tell when a cell is in sound condition and to measure deviations from the normal as well as changes due to stimulation. They also permit us to analyze mathematically the processes of injury, recovery, and death (63).

Since large plant cells offer certain marked advantages for these studies this review will be largely limited to investigations carried out on such cells.

The chief topics to be treated are the current of injury, the current of action, electrical resistance and capacitance, and the nature of the protoplasmic surface.

THE CURRENT OF INJURY. 1. *Protoplasmic potentials.* The current of injury is measured by leading off from an intact spot to another region of the same cell where the P.D. has been reduced approximately to zero by killing it.

The cells here described have a large central vacuole surrounded by a thin layer of protoplasm and the full value of the current of injury measures the P.D. across this layer of protoplasm. At the inner and outer surfaces of the protoplasm are non-aqueous layers which are the chief seats of the observed P.D. Between them lies an aqueous phase.

2. *Unlikeness of inner and outer surfaces.* When we place *Valonia* sap outside a *Valonia* cell and lead off from it to a killed spot or to a capillary which passes through the protoplasm into the vacuole, we have the chain

$$\text{sap outside} \mid \overbrace{X \mid W \mid Y}^{\text{protoplasm}} \mid \text{sap in vacuole}$$

where X and Y are the non-aqueous surface layers and W is the aqueous layer of the protoplasm. If X and Y were alike this should give no E.M.F. but we actually find about -65 mv. for *Valonia macrophysa* (30). Similar experiments give for *Halicystis Osterhoutii* $+50$ mv. (5) and for *Halicystis ovalis* $+60$ mv. (12). (The plus sign means that the positive current tends to flow from the sap across the protoplasm to the external solution: the minus sign signifies the opposite direction.)

Further evidence is found in the experiments of Blinks who kept cells alive when pierced by two capillaries serving for the entrance and exit of various solutions which he substituted for the sap of the large central vacuole (14). In *Halicystis ovalis* and in *H. Osterhoutii* K^+ produces a large potential effect with the outer surface but not with the inner. In these experiments cells could be kept alive when their sap had been replaced by that of another species (of different composition) or by sea water.

These experiments show that great differences can exist between X and Y (66, p. 1014) and this is confirmed by finding that the properties of X can be profoundly altered without affecting Y (82a).

The P.D. across the protoplasm is probably due in part to ions in W which have very different mobilities in X and Y (66, p. 1014). It is somewhat surprising to find such great differences on opposite sides of a layer of protoplasm less than 10 microns in thickness (66, p. 1021) and it would be interesting to discover the mechanism by which they are created.

3. *Positive and negative currents of injury.* Under normal conditions differences between the sap and the external solution influence the current of injury. Thus in the case of *Nitella*, where X and Y do not seem to differ greatly, since the P.D. across the protoplasm when sap is placed outside is only -15 mv. (73), the current of injury depends on what is placed outside. When the cell is growing in pond water the current of injury is $+100$ mv. or more, owing chiefly to the fact that the sap has about 0.05 M potassium and the external solution contains very little.

Most of the potential drop appears to take place across Y. If Y has a thickness of 0.1 micron the potential drop across it may possibly amount to as much as 10,000 volts per centimeter (67).

With cells growing in pond water the P.D. across X appears to be small because the concentration gradient of K^+ across X is small.

The net result is a P.D. across the protoplasm of 100 mv. or more.[1]

[1] Organic ions in W may play some part (66, p. 1015).

Hence the current of injury is negative as with muscle and nerve, *i.e.*, the injured part is negative in the external circuit. But when we place 0.05 M KCl outside the cell the P.D. across *X* becomes negative so that the net P.D. across the protoplasm is −15 mv. and in consequence the current of injury becomes positive.

With *Halicystis Osterhoutii* growing in sea water the current of injury is negative (70 mv. (5)); but it can be made positive by applying KCl (12) or NH_3 (13); also by 0.6 M NaCl (18) or by an outgoing electrical current (15). With *Valonia* growing in sea water the current of injury is about 10 mv. positive (66, p. 1014) (29) (30) (31).

In muscle and nerve the current of injury seems to depend chiefly on K^+ (28) (36) (37) (50) (51) and can be diminished by applying K^+ to the outside though it has not yet been possible to reverse it as in plant cells.[2] Under certain circumstances K^+ appears to escape and influence surrounding cells (56a).

To what extent the situation in muscle and nerve corresponds to that in *Nitella*, with two protoplasmic surfaces, is uncertain. Unpublished results of W. L. Francis indicate that when freshly crushed muscle (or a solution resembling it) is applied to the exterior of the muscle fibre, the current of injury is not zero and in this respect the situation recalls that in *Nitella*. If the surface of the cut fibre were promptly regenerated (as in *Ameba*) there would be no current of injury. The question arises whether the interior of the muscle fibre corresponds to a vacuole filled with a gel in which the fibrillae are embedded (in which case regeneration of the protoplasmic surfaces would not interfere with the current of injury any more than it would in *Nitella* since the circuit would pass only once through living protoplasm).

It may be added that the two surfaces of the protoplasm in *Nitella* enable us to explain the forms of the action current, including some which resemble those found in nerve (54).

The current of injury in nerve may be affected by the supply of oxygen (28) (47) (50). This may depend on the ions formed in metabolism or on changes in the non-electrolytes composing the protoplasmic surface. Unpublished results of W. L. Francis show that in *Nitella* the oxygen tension can be reduced to a very low value without producing much change in the current of injury.

[2] To some extent this may depend on the fact that only the outer fibres of the muscle or nerve are directly accessible to applied K^+; hence if the reversal occurred in these it might be swamped by the effect of the inner fibres. The intact inner fibres also reduce the value of the concentration effect by acting as short circuits (76).

Let us now consider the mobilities of ions in the protoplasmic surface.

4. *Ionic mobilities.* In studying these cells it has been found advantageous to proceed as if the P.D. were chiefly due to diffusion potentials.[3] Only thus are we able to predict satisfactorily the P.D.s produced by various mixtures of ions.

On this basis we find that the apparent mobilities in the non-aqueous surface layers differ greatly from those found in water. If we take that of Cl^- as unity we find in *Valonia* the apparent mobility of K^+ is 20 and that of Na^+ is 0.2 (31). In *Nitella*[4] the value for K^+ is 85.45 and that for Na^+ is 2.18: hence when we lead off from a spot in contact with 0.01 M KCl we observe a P.D. of 85 mv. or more. In general the inorganic anions do not appear to differ much from Cl^- but the guaiacol ion shows a very high mobility in *Valonia.*

Why do these values diverge so much from those found in water? The experiments indicate that in these cells (as in many others) the protoplasmic surface consists of a liquid layer with a low dielectric constant. Kraus (60) has shown that in such media charged complexes are formed. Hence if the external solution contains K^+ we may have in the protoplasmic surface not only K^+ but also such charged complexes as $(KX_I)^+$, $(KX_{II})^+$, where X_I and X_{II} are elements or radicals.

Owing to the formation of such complexes the diffusion potential of KCl might seem to be much greater than that of NaCl in the protoplasmic surface. In consequence the calculated mobility of K^+ might appear to be much greater than that of Na^+. This may explain the following facts.

We find the following mobility ratios (taking the mobility of Cl^- as unity):

In water	K − Na =	0.99 ÷ 0.66 =	1.5
In *Nitella*	K − Na =	85.45 − 2.18 =	39.2
In *Valonia*	K − Na =	20.0 − 0.2 =	100

[3] Phase boundary potentials may cancel out to such an extent as to have no great effect (64) and theoretically would not be expected to give a concentration effect. Donnan potentials are small in the presence of much electrolyte and can be calculated only at equilibrium which seldom occurs as long as metabolism is going on. The Donnan potentials with a salt like KCl would decrease as the concentration increased which does not correspond to the behavior of the potential with living cells.

[4] The mobilities of H^+ and Rb^+ are high: those of Ca^{++} and Mg^{++} low; NH_4^+ is intermediate but the value seems to fluctuate a good deal, depending on the condition of the cell; that of Cs^+ is less than that of Rb^+.

(likewise in muscle and in nerve the mobility ratio K ÷ Na may have a high value.)

As such differences are not explainable by solvation[5] or by the partition coefficients of simple ions, it would seem that they can be accounted for only by the formation of charged complexes.

Changes in such complexes might well be brought about either by removing substances from the protoplasmic surfaces or by adding them to it. This might explain the change of apparent mobilities from K > Na > to Na > K in *Nitella* by treatment with distilled water (68) and from K > Cl > Na to Na > Cl > K in *Valonia* by treatment with guaiacol (70). (The cells are not injured by these two methods of treatment.)

5. *The behavior of potassium.* The question arises, how can potassium, which causes an inwardly directed (negative) P.D. when applied outside, be responsible for the outwardly directed (positive) P.D. observed in *Nitella*? Some light on this question is obtained by the study of models.

In these models a solution of 0.05 M KOH is used as the external phase and a mixture of guaiacol + *p*-cresol is used to represent the non-aqueous protoplasmic surface. The P.D. may depend on two things: 1, K^+ reaches a higher concentration inside than outside, and 2, K^+ may enter paired with an ion of relatively high mobility and so set up a relatively small potential directed inward but in leaving the cell it may be paired with an anion of lower mobility and so set up a relatively high potential directed outward.

Using a model (65) in which 0.05 M potassium guaiacolate (called K*G*) penetrates through guaiacol (representing the non-aqueous protoplasmic surface) to an aqueous phase containing CO_2 (representing the cell sap) we observe at the start of the penetration an inwardly directed P.D. of about 40 mv. apparently due mostly to the higher mobility of K^+ in the non-aqueous phase as compared with G^-. As K*G* penetrates it forms $KHCO_3$ in the artificial sap, reaching a concentration of 0.6 M or more, and as K^+ has a higher mobility than HCO_3^- in the non-aqueous phase there is an outwardly directed potential of about 80 mv. The net result is 40 mv. directed outwards. Water and K*G* then penetrate at about the same rate so that the volume of the "artificial sap"

[5] In water Cs > Rb but the opposite is found in *Valonia*, in *Nitella*, and in liquid ammonia. The situation in liquid ammonia is regarded by Kraus (60) as due to solvation but the effect in *Valonia* and *Nitella* is perhaps too great for such an explanation.

increases while its composition and outwardly directed P.D. remain approximately constant. This seems to be analogous in some respects to what happens in *Nitella*, where potassium enters until its concentration inside becomes much greater than outside (66, p. 981).

6. *Currents of injury in tissues.* Before leaving this subject attention may be called to certain complications found in tissues. If we cut off the end of a *Nitella* cell a compression wave passes quickly down the cell initiating a death process at each point it touches. When the death process is complete the P.D. of the whole cell is reduced approximately to zero but while it is still going on the differences between the two ends of the cell produce the current of injury which, however, does not last long (74) (75). In muscle and nerve the death process is slow (perhaps because they are less turgid) so that the current of injury lasts a long time.

A lasting P.D. closely resembling the current of injury is observed in *Nitella* when we employ two cells in their natural union and cut one of them. As soon as the cut cell dies sap begins to diffuse out and when it has passed through the intervening cell wall and has reached the protoplasm of the next cell it changes its P.D. from 100 mv. or more positive to a few mv. negative and this effect can persist for a relatively long time. It might easily be mistaken for the current of injury if it took place in a tissue (77).

THE CURRENT OF ACTION. 1. *The typical action curve.* In *Nitella* the usual course of events is as follows: *a*, a loss of P.D. which is often complete and may amount to 200 mv. or more. In consequence the action curve shows a sudden upward movement which very often goes to zero. This is easily seen in *Nitella* by leading off from an intact spot to one whose P.D. has been reduced approximately to zero by chloroform or by KCl (67). This part of the action curve (called for convenience the *o* movement) corresponds to the "spike" in the action curve of nerve.

A simple way to explain this movement is to assume that it is due to the movement of K^+ (fig. 1). Before the action current starts the concentration of K^+ is much higher on the inside of *Y* than just outside it (in *W*). Consequently there is a P.D. of 100 mv. or more across *Y* but there is no flow of current because neighboring regions have the same P.D. When a neighboring region loses its P.D. a flow can take place just as when 100 mv. (D.C.) are applied from an external source (this is usually sufficient to stimulate).

When a potential is applied from an external source we find a threshold below which no action current is produced no matter how long the

flow continues. It therefore seems probable that to produce the loss of P.D. the non-aqueous layer *Y* must be temporarily broken down or made permeable by the application of sufficient E.M.F. (evidence of which

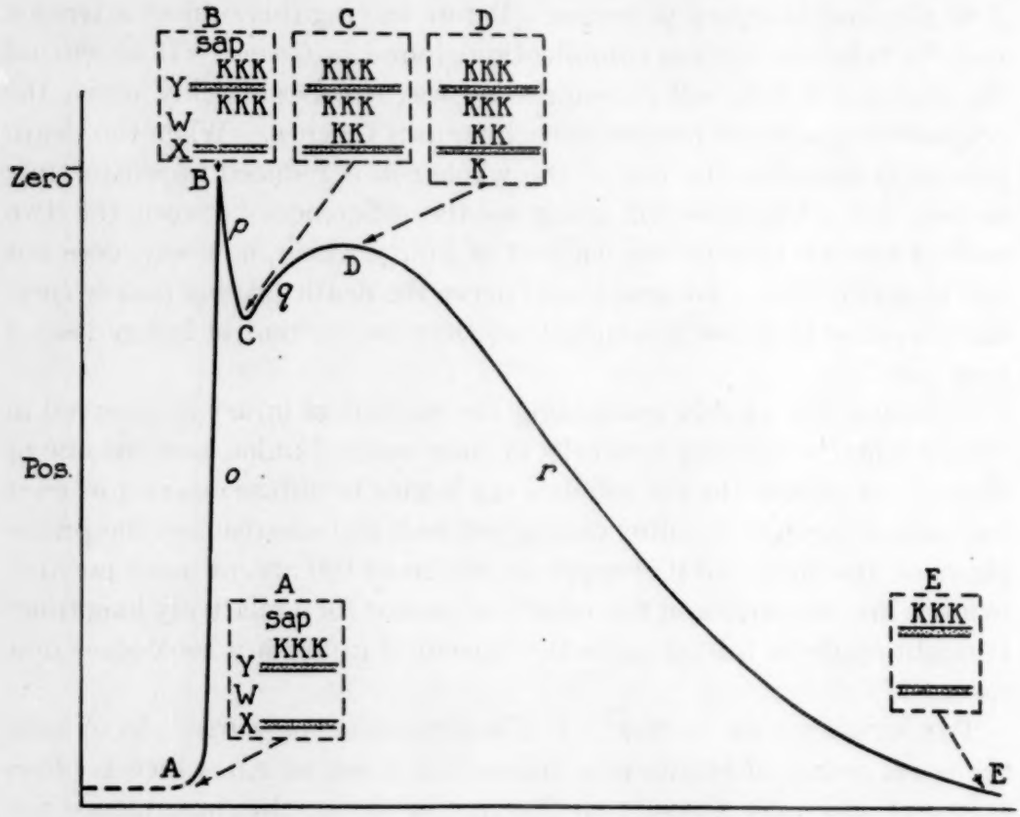

Fig. 1. The unbroken line shows changes in P.D. during the action current in *Nitella*, supposedly due to the outward movement of potassium. The broken line shows the P.D. in the resting state, before the outward movement of potassium begins.

In the diagrams the symbol K denotes the outwardly moving potassium (reduction in its concentration is shown by reduction in the number of symbols). Each stage of its progress is marked by a change in P.D.: for example, in diagram A the observed P.D. is due to the relatively high concentration of potassium at the inner surface of *Y*; in diagram B we see that potassium has reached the outer surface of *Y* and in consequence the P.D. has disappeared.

The duration of the action current is usually about 15 seconds. The rate of transmission is of the order of 2 to 20 cm. per second (17).

is seen in the sudden drop in resistance occurring at this time (7)). When[6] this happens K^+ presumably moves outward freely, producing

[6] If *Y* is only 0.1 micron thick the application of 100 mv. will produce a potential drop of about 10,000 volts per centimeter across *Y*.

the p and q movements which will be discussed presently.[7] In accordance with this conception loss of P.D. (*i.e.*, stimulation) occurs with an outgoing but not with an ingoing current (since the latter would not carry K^+ outward). This is in harmony with the local circuit theory (57) (61).

It follows that when the P.D. of a spot A has fallen to zero (or to a low value) because of an outgoing current, a neighboring spot B cannot discharge into it with sufficient flow of current for propagative purposes if its P.D. has been too much reduced by injury. But if a salt bridge (consisting of cotton moistened with an appropriate salt solution) connects A to a normal spot C on the other side of B a current can flow from C to A and thus reduce the P.D. at C to zero: the next spot D can then discharge into C and so the negative variation can continue to travel along the cell. This has been observed (79) and is a good proof of the local circuit theory (61) since it seems inexplicable on any other basis.

It likewise follows that anything that interferes with the breakdown of Y or its increase in permeability will inhibit stimulation. We shall have occasion to consider this in connection with anesthesia (p. 230).

On this basis we should expect a mechanical rupture of Y to cause a sudden loss of P.D., as appears to be the case. Pinching or bending a *Nitella* cell produces a compression wave which travels very rapidly along the cell and causes a loss of P.D. at each spot it reaches (p. 228). This effect is increased by cold, perhaps because Y is thereby made more susceptible to rupture: possibly stimulation by cold (53) may be due to a rupture of Y by protoplasmic movement when Y is in a susceptible state.

It may be added that Blinks (13) in view of the effect of NH_3 on *Halicystis* has suggested that the action current of *Nitella* may be associated with changes of intracellular pH, in line with the ideas of Bethe, although he casts doubt (11) on the supposed experimental basis of Bethe's views (color changes of anthocyan in cells).

b. The p movement. When Y is temporarily broken down or otherwise rendered permeable K^+ can pass out freely into W and when it comes in contact with X it can set up an outwardly directed potential, thus restoring a part of the original P.D.

[7] A breakdown or short circuit of Y by means of leaks could cause a loss of P.D. without any movement of K^+ but if there were no such movement we should expect a much more rapid recovery than actually occurs and we could not account for the p and q movements. The rise in temperature observed by A. V. Hill (50) might result from the reactions due to substances brought together by the increased permeability of the protoplasmic surface.

This is a regular feature of the action current in *Nitella* under normal conditions. But in cells treated with distilled water K^+ can no longer set up much potential when it comes in contact with X and we then find little or no p movement (86). This condition is regularly found in *Chara* (67). But when X is made sensitive to K^+ in *Chara* there is some p movement. *Nitella* cells in which the outgoing action current flows lengthwise chiefly in the protoplasm (so that K^+ does not come in contact with X) show little or no p movement (85).

c. The q movement. As soon as K^+ reaches the outside of X the positive P.D. built up during the p movement will tend to disappear because it depends on the gradient of K^+ across X. This loss of positive P.D., carrying the action curve back toward zero, is called the q movement.

Most of the K^+ which comes out in this way presumably moves back promptly, as described in the next section. An attempt to detect the exit of electrolyte was made by Sen (90) but the increase in conductivity noted by him may have been in the protoplasm rather than in the film just outside it as Blinks (7) finds that a marked decrease in effective resistance accompanies the action current.

The q movement is often much reduced and thus gives rise to curves resembling those found in nerve (54).

d. Recovery (the r movement). As soon as K^+ has moved to the outside of X, thus reducing the P.D. approximately to zero, it would appear that the forces[8] which normally carry K^+ into the cell move it back again into the sap, thus making the process reversible. It is sometimes stated that there is a permanent loss of K^+ from nerve during the action current but it would seem that this can hardly be considered normal since if it were the action current would not be a wholly reversible process.[9]

Anything which interferes with the return of K^+ to the sap will correspondingly interfere with recovery. Ordinarily recovery requires about 15 seconds, but it can be greatly shortened (*e.g.*, by treatment with NaCl) (85) or lengthened (*e.g.*, by treatment with distilled water) (68).

This explanation of the action curve seems to be useful as a working hypothesis since it accounts not only for normal behavior but also for deviations (85). One objection to it has been discussed by Blinks (7). This is that recovery can occur while an outgoing electric current is

[8] Regarding these forces see (66, p. 982) (72).

[9] It is, of course, possible that unusual circumstances might cause a delay in the return of potassium, and that excessive stimulation should cause potassium to be lost more rapidly than it can be replaced.

being applied from an external source. It is possible that this may be due to changes in permeability. Although at first the outward current appears to make Y more permeable to K^+ it would not be surprising if, as the current continued to flow, this effect should diminish as the result of the movement of substances[10] or of changes in hydrogen ion concentration (as in the experiments of Bethe and Toropoff (2) (3)) or of other alterations in metabolism. If, as a result, the permeability of Y to K^+ should diminish somewhat[11] while the current is flowing outward the concentration of K^+ would increase just inside Y and diminish just outside Y. This would produce an outwardly directed (positive) potential which might increase until equal to that in the resting state, thus producing recovery. It might go further and produce a greater positive potential, resembling the "after potential" found in nerve (45) (46).

With such a flow of current from an external source or with an action current "positive after potential" (which seldom occurs in *Nitella*) might arise in several ways, *e.g.*, *1*, as the result of a temporary increase in the concentration of K^+ just inside Y due to a change in permeability; *2*, the movement of organic cations into W as the result of the action current. These might increase the positive potential by having different effects on Y and X. Such substances are apparently responsible for some of the positive P.D. in *Nitella* and for a good deal of the positive P.D. in *Halicystis* (12). Such substances might not persist long in W since they would tend to pass out by diffusion or to be used up in metabolism. *3*. There may be a temporary increase in the mobility of K^+ or Na^+ due to changes in the composition of X or Y produced by the action current.

When the current is interrupted before recovery is complete the process of restoring the positive potential of the resting state will continue because potassium[12] will be moved into the sap from W by the forces (66, p. 982) which normally produce this movement in the resting state of the cells.[13] This will be aided by the ingoing current which according to the local circuit theory occurs during recovery.[14]

[10] Such a movement (of R) appears to change X when an action current restores the potassium effect (86).

[11] This change need not be sufficient to affect the flow of current greatly.

[12] In this process potassium presumably moves chiefly in molecular form through Y. (*Cf.* (66, pp. 994, 1003).)

[13] This will be aided by the return of the normal mobility of K^+ in Y and consequently of a higher outward diffusion potential.

[14] This will depend somewhat on the position of the spot. If the action current proceeds from left to right a spot at the extreme right end of the cell will not receive much ingoing current of this sort.

An objection to the idea that the action curve is due to the outward movement of cations is voiced by Gasser (45) (46) on the ground that a sub-threshold current which would carry ions outward fails to produce an action current. This might be due to two causes: *a*, potassium tends to move inward in the resting state and the forces causing this must be overcome[15] before K^+ can travel outward; *b*, there must be sufficient outgoing current to produce the necessary increase in permeability. One way of testing this is to employ cells in which an applied E.M.F. produces no action current and presumably fails to produce an increase of permeability (such cells can be obtained by treatment with distilled water and they occur naturally during the summer (82a)). With these cells an outgoing current, normally adequate for stimulation, produces only a gradual loss of P.D. such as would be expected if K^+ moved slowly outward through protoplasm whose permeability remained normal (unpublished results).

2. *Unusual forms.* Before any one movement is over, the following movement may have begun so that the timing of the various movements as well as their magnitude affects the result. Hence the action curve shows a great variety of forms (54). Some of these can be produced experimentally. The time of recovery is especially easy to influence experimentally, *e.g.*, by alcohol (82).

Great variation is found when irritability is restored after treatment with distilled water. Recovery is often slow and unusual forms of the action current are more apt to occur.

Cells soaked in 0.01 M NaCl, to make the protoplasm more conductive, show a great variety of action currents, mostly with single peaks[16] similar to those which occur spontaneously (85) and which have been explained as the result of unusual conductivity of the protoplasm. In these cases there seems to be considerable variation in the amount of K^+ in *W*.

If the concentration of K^+ in *W* is not exactly the same before and after the action current it may affect the next action current. If it increases with successive action currents[17] there may be a corresponding

[15] An example of this is seen in certain models (9).

Even in cells in which potassium no longer moves in the resting state but has reached an approximate equilibrium (as might happen in cells which were not growing) energy will be required to upset this equilibrium and cause K^+ to move outward.

[16] S. E. Hill, unpublished results.

[17] The effect of increase of K^+ in *W* will depend on whether it is in contact with *X* as well as its effect on the potential of *X* and *Y*. It might conceivably happen

decrease in the resting P.D. and in the spike (*o* movement) of the action curve (and *vice versa* (85)). This appears to happen more frequently in cases where the outward lengthwise flow of the action current takes place in the protoplasm rather than in the cellulose wall: in such cases a variety of action curves is observed (85).

In some cases action currents produce after-effects which can be detected only by special means, *e.g.*, by the application of K^+. For example, K^+ in external contact with *X* no longer produces its characteristic negativity with *Nitella* cells which have been exposed to distilled water. Such cells in contact with 0.01 M KCl may show a positive P.D. of over 100 mv. (instead of 2 or 3 mv. as in normal cells), and may give action currents. But during an action current when K^+ moves out from the sap it would seem that *R*, the substance responsible for the potassium effect, also moves out and at the close of the *o* movement the K^+ in external contact with *X* produces its usual negativity, reducing the total P.D. approximately to zero. There is therefore no recovery (86).

If 0.01 M NaCl is in contact with the cell this after-effect of the action current is not perceived for recovery takes place as usual, but if 0.01 M KCl is then applied the P.D. falls approximately to zero.

In *Chara* this state of affairs is the normal one. This may be due to the fact that ordinarily *R* is dissolved out of the surface so fast by the pond water that K^+ does not show a strongly negativating effect until a larger supply of *R* is carried into the surface during the action current. This is also true of *Nitella* during the summer.

It seems quite possible that a variety of after-effects of action currents occurs which may escape detection if the cell is at rest under normal conditions. Some of them involve a change in the threshold. In this connection the increase of efficiency observed during the early stages of warming up on the running track or in memorizing lists of words may be of interest.

In concluding this section, attention may be called to the fact that three very striking phenomena accompany the action current. 1. A loss of the P.D. across the protoplasm (*i.e.* loss of the demarcation current): this is usually complete but may be only partial. 2. A disappearance of the back E.M.F. (p. 232) which is observed in the resting state when an external E.M.F. is applied. 3. Due really to 2, a large de-

(85) that K^+ in *W* might increase the outward (positive) potential of *X* to simulate recovery. This would, however, not restore the normal resistance and polarization and this may explain the prolonged loss of resistance noted in some cases by Blinks (7).

crease (amounting to about 90 per cent) in the effective electrical resistance (p. 222).

As all of these effects can be produced by the application of 0.01 M KCl to the exterior of the cell in the absence of any action current, it seems reasonable to assume that potassium moves from the sap to the exterior during the outward flow of the action current and that, to a considerable extent, this movement is responsible for these phenomena.

3. *All or none.* In a typical action current there is a complete loss of P.D. and if the "all or none" law held this might be expected in all cases. It often happens, however, that only a partial loss occurs and it may amount to from 2 to 99 per cent of the total P.D. across the protoplasm.

In some cases this might be explained as a complete loss of the P.D. across Y (85) (but not across X) so that in this layer the process would obey the law of "all or none." But in many cases there is no ground for such an explanation, especially where the loss is very small. A partial loss of P.D. as the result of electrical stimulation can be produced experimentally, *e.g.*, by ethyl alcohol (82). In stimulation by cold (53) the loss is usually incomplete.

It is of interest to note that in general the loss is at first slow and then rapid as though two stages were involved (81): this is well illustrated in action currents started by alcohol (82). The first stage involves a rather slow partial loss and if the process did not go beyond this stage the result might be that found in those cases where the all or none law does not appear to hold.

When the loss is a large fraction of the total it might be due in some cases to the fact that the p movement begins before the o movement ends (54).

The loss of P.D. appears to depend on two things: 1, a breakdown or short-circuiting due to leaks in the non-aqueous layers; 2, the movement of potassium across these layers. There is no theoretical reason why either of these processes should lead to a complete loss of P.D. in all cases.

On the other hand there is no theoretical reason why conduction with a decrement should occur nor has it been definitely established (it occurs with the mechanical variations mentioned in the following section).

4. *Mechanical negative variations.* When a *Nitella* cell is cut or pinched a compression wave travels rapidly along the cell causing a loss of P.D. at each point it reaches. It can pass a killed spot and in this respect differs from an ordinary propagated negative variation; it

likewise shows a decrement in passing along the cell. It can in some cases produce as it travels an ordinary stimulation, resulting in a propagated negative variation (74) (75) (80) (81).

5. *Positive variations.* Positive variations can be produced by placing a *Nitella* cell in contact with 0.05 or 0.1 M KCl, thereby creating a negative P.D. of about 30 mv. When such a cell is pinched a compression wave travels along it, producing a loss of P.D. at each spot it reaches. The result is that the spot becomes 30 mv. more positive (as its negative P.D. of 30 mv. disappears). After recovery another pinch produces a second positive variation (83). These positive variations are not propagated as ordinary negative variations are, since they result from mechanical variations as described in the preceding section.

6. *Pacemakers.* When the P.D. at any spot is reduced to zero, *e.g.* by chloroform (78), by alcohol (82) or by KCl, a flow will start from a neighboring region[18] thereby producing an outgoing current and a loss of P.D. at that spot: when it recovers its P.D. a new flow can start and thus a series of successive action currents may arise. The treated spot thus becomes a pacemaker.

The rhythm can be regulated by various means. It can be slowed down by increasing the refractory period and hastened by soaking the cell in 0.01 M NaCl[16] which makes the protoplasm a better conductor (85).

Pacemakers may arise spontaneously in the cell and may subsequently shift their positions.

As a rule the entire cell follows the rhythm of the pacemaker but occasionally a spot skips every other impulse (55), thus recalling auricular flutter of the heart.

These observations are of especial interest in view of the significance of pacemakers in biology and psychology. This has recently been discussed by Hoagland (56).

7. *Anesthesia.* Experiments on *Nitella* throw an interesting light on some aspects of anesthesia. Under normal conditions cells produce action currents when stimulated by a direct current of 80 to 200 mv. But after exposure for 2 or 3 days to distilled water this is no longer true and the highest D.C. voltage that can be applied without injury (about 500 mv.) does not stimulate (82a).

The experiments indicate that the irritability of the cell depends upon a substance (or upon a group of substances) which for convenience may be called *R*. Under normal conditions this is produced as rapidly as it

[18] Provided, of course, that the potential drop along the cell is sharp enough to overcome the resistance and produce sufficient flow (79).

leaves the cell but in distilled water it is dissolved out more rapidly and a deficiency results which leads to loss of irritability. When the cells are returned to their normal environment of pond water or to other solutions containing calcium the irritability returns, presumably because the dissolving action is checked and the concentration of R rises sufficiently to permit the return of irritability. Such experiments suggest that the rôle of calcium in antagonism may consist, to some extent at least, in checking the dissolving out of substances from the surface (52). That a dissolving action is responsible is shown by the fact that the water in which cells have been standing contains organic substances which, when suitably concentrated and applied to the cell, quickly restore irritability.[16]

Such substances appear to be widely distributed as might be expected in view of the general occurrence of irritability. For example, when irritability has been lost it can be restored in a few seconds by treatment with human blood, urine, or saliva, or with other mammalian bloods; also with white of egg and with milk (84). If the substances causing this effect are responsible for the irritability of nerve and lead to disturbances in nervous functions when deficient, such substances must play an important part in ontogeny and in phylogeny as well as in pathology.

These experiments suggest that some anesthetics may produce their effects in the same way as distilled water, *i.e.*, by removing substances from the protoplasm.

What is said of irritability applies also in general to the potassium effect, *i.e.*, the large P.D. (about 85 mv.) observed in leading off from a spot in contact with 0.01 M KCl to one in contact with 0.01 M NaCl. (In this respect the cell acts somewhat like a potassium electrode.) The loss of this property is an additional indication of the profound change in the protoplasmic surface caused by dissolving out R.

What is the nature of R? One way of attacking this problem is to observe the effect of various pure substances. We find that the following can restore irritability or the potassium effect or both: NH_3, NH_4Cl, tetraethyl ammonium chloride, guanidine, adrenaline, and ephedrine. In some cases only one effect was secured and when both were obtained one was usually restored before the other (84). This indicates that they depend upon somewhat different conditions.

It would not be surprising to find that a variety of substances are effective. We should expect that any substance capable of facilitating the breakdown or increase of permeability of the non-aqueous protoplasmic surface under an applied E.M.F. would tend to restore irritability.

Any substance which increased the apparent mobility of the potassium ion as compared to that of the sodium ion (*e.g.*, by forming complex ions) or which increased the concentration of potassium ions in the non-aqueous layer (*e.g.*, by chemical reaction) would probably tend to restore the potassium effect.

ELECTRICAL RESISTANCE AND CAPACITANCE. 1. *Electrical resistance.* These large plant cells offer special advantages for the study of electrical resistance because we can estimate how much of the current passes through the protoplasm and how much through the cell wall.

In *Valonia* (6) growing in sea water the D.C. resistance of the protoplasm is about 10,000 ohms per square centimeter; in *Nitella* (7) and in an uncorticated *Chara*, both in contact with pond water, about 250,000 ohms per square centimeter.

The resistance depends on the solution in contact with the cell. Thus in *Nitella* the resistance falls off but little when the tap water at the contacts is replaced by 0.1 M NaCl; nor do similar concentrations of LiCl, CsCl, NH_4Cl, $CaCl_2$, or $MgSO_4$ greatly decrease the value. But when 0.1 M KCl is applied the resistance drops from 250,000 to 10,000 ohms per square centimeter. RbCl has a similar effect (this is paralleled by the large effect of these salts on P.D.[4])

In other words, the conductance of the protoplasm of *Nitella* in contact with 0.1 M KCl is at least 25 times as great as when in contact with 0.1 M NaCl. This is in good agreement with the values of the apparent mobilities (p. 220) which can be employed in the formula

$$\frac{\lambda_{KCl}}{\lambda_{NaCl}} = \frac{U_K + V_{Cl}}{U_{Na} + V_{Cl}} = \frac{85 + 1}{2.2 + 1} = 26.9$$

With an outgoing current (above a certain threshold value, which may sometimes be exceeded by applying as little as 15 mv.) a negative variation occurs accompanied by a temporary decrease of resistance (7) and of P.D. which may fall as low as when 0.05 M KCl is applied outside. This might be due to the movement of K^+ from the sap into the protoplasm or to the temporary breakdown or increase of permeability of the non-aqueous layers.

2. *Capacitance and polarization.* The resistance discussed above (as measured with direct current in a Wheatstone bridge) is largely due to a back E.M.F. developed during the flow of current: in other words the protoplasm acts largely as a condenser or as a polarizing electrode.

The effects produced by a flow of current in *Valonia*, *Halicystis*, and *Nitella* have been studied by Blinks (6) (7) (8) (10) (also articles in press).

The results indicate that the protoplasmic surface is a non-aqueous layer[19] of low conductivity and not equally permeable to all ions. This could produce static capacity,[20] since it is a very thin insulator, and polarization capacity,[21] due to its unequal permeability to ions.

The results indicate that both may be involved. They may be illustrated as follows. When *Valonia macrophysa* is impaled on a capillary an ingoing or outgoing current can be sent through the protoplasm. In the "regular" state of this cell a current in either direction causes prompt development of a partially or completely reversible counter E.M.F. directly proportional to that applied. It usually requires about a second for the counter E.M.F. to reach its full value, and a similar time to disappear when the applied current ceases.

The curves for "charge" and "discharge" resemble those of a condenser or of a polarizing electrode. The apparent capacity of *Valonia* is of the order of 1 microfarad per square centimeter and that of *Nitella* about the same, although this value is difficult to assign with assurance because the curve is not affected by inserting series resistance as it would be with a condenser.

When alternating current is used the capacitance and resistance of *Valonia macrophysa* fall off as the frequency increases. After correcting for the effects of the cell wall and of the sap Blinks finds that the equivalent series resistance of the protoplasm is nearly proportional to the reciprocal of the frequency. In this respect the behavior of the protoplasm resembles that of platinum electrodes in a solution of KCl.

All this applies to the cells in what may be called the "regular" state. There is also a "delayed" state where the counter E.M.F. develops more slowly and irregularly (with an S-shaped curve) and is not directly proportional to the applied E.M.F.

Inward currents have a "conditioning effect" and can restore or alter the surface and so make polarization possible. Outward currents may destroy the polarizability (and possibly the surface).

Cells can be changed from the "delayed" state to the regular state by treatment with *p*-cresol (and certain other acids) but ammonia has the opposite effect. In view of this it seems possible that ingoing current tends to make the part of the protoplasm concerned more acid and outgoing current more alkaline.

[19] The hypothesis of a pore system of a monomolecular layer might explain many of the electrical measurements but would be in conflict with certain facts (p. 233).

[20] *I.e.*, like that of mica which does not change with frequency.

[21] *I.e.*, changing with frequency.

Increasing the external concentration of K^+ has little effect on polarization in *Valonia* but greatly reduces it in *Nitella* (in both plants it makes the P.D. much more negative).

In these cells the current can be made to pass only once through living protoplasm and the effects of inward and outward currents can be studied separately. Tissues and cell suspensions present a somewhat different situation in that many protoplasmic surfaces are in series. In such cases there is good agreement between measurements made with direct current and with low frequencies (as shown by McClendon for erythrocytes (62) and by Blinks for *Laminaria* (4)). Measurements at low frequency on *Laminaria* (63) show that the resistance is a good indicator of normal condition and that injury and recovery may be partial or complete and can be predicted by the use of suitable equations. Similar changes may occur in other plants (35) and have been observed by Blinks in single cells of *Valonia*.[22]

Measurements made on *Laminaria* lead to the idea of a steady state due to consecutive reactions: this has been discussed by Hoagland (56) in connection with certain basic concepts of biology and psychology.

The results with skin (48) (49), muscle (40) (19), nerve and certain other cells (22) (23) indicate that polarization capacitance plays the chief rôle (19) (20) (22).

Measurements on suspended cells indicate that the protoplasmic surface has a low conductivity and that the capacitance is chiefly static (independent of frequency) of the order of 1 microfarad per square centimeter. This applies to red (41) and white (43) blood cells, yeast (42) and to the marine eggs *Asterias* (26), *Arbacia* (27), and *Hipponoë* (25).

NATURE OF THE PROTOPLASMIC SURFACE. The results just mentioned indicate that the protoplasmic surface has a low conductivity, low dielectric constant, and no great thickness. Where the capacitance is chiefly static it might be inferred that there is a monomolecular layer (39) (33) (34). This supposes a dielectric constant of about 3 for the surface.

In *Valonia* and *Nitella* the conception of a monomolecular layer meets with great difficulties. Not only is the capacitance of a different type, being chiefly polarization rather than static capacitance, but the surface is evidently inhomogeneous and consists of a mixture of substances. This is shown by the fact that its properties can be radically changed by removing substances from it (p. 229) or adding them to it (p. 220) and also by the fact that such substances as ammonia (67a) and guanidine (58) enter *Valonia* by combining chemically with one or more constituents of

[22] Unpublished results.

the surface. Furthermore if the surface were monomolecular and its charge determined the entrance of electrolytes we could not have the situation found in *Valonia* where the surface appears positively charged in respect to NaCl but negatively charged in respect to KCl[23] (30) (32).

The fact that the apparent ionic mobilities are so different from those found in water shows that the surface is non-aqueous and other experiments show that in these and in many other plant cells it is a liquid layer (66, p. 1003) so that we cannot regard it as a "pore system" unless perhaps we suppose that a monomolecular layer exists at the surface of the liquid which gives it the properties of such a system. But in that case the properties of the surface layer would depend not only on the monomolecular layer but also on the non-aqueous liquid layer upon which the monomolecular layer rests.

These conceptions of the protoplasmic surface are also confirmed by studies on permeability (66, p. 1010) which show that the surface is non-aqueous and does not resemble a protein film imbibed with water. It might be thought that a non-aqueous layer immiscible with water could not admit sufficient water and strong electrolyte to supply the needs of the cell but experiments with models have demonstrated that this presents no difficulty (88) (89).

In conclusion it may be well to call attention to the variability of the protoplasmic surface. This is shown by connecting two regions of a *Nitella* cell[24] which are in contact with the same solution: this seldom shows exactly zero P.D. and may give 30 mv. or more. Sometimes we and observe rapid changes in the P.D. (85).

This might depend on the condition of the protoplasm or on the ions in contact with it. Both of these factors may be influenced by metabolism. In some cases the condition of the protoplasm alone seems to be responsible as when we find that the concentration effect (64) or the potassium effect (68) or the first part of the action curve is variable (54).

The condition of the protoplasm may be reversibly changed by a variety of methods (66, p. 1016), including temperature (53), lack of calcium (5) (63), anesthetics, alcohol (82) distilled water (p. 229), guaiacol (p. 220), ammonia (13) (68), guanidine (58), adrenaline (p. 230), blood other animal products (69).

CONCLUSION. In concluding an account of these results attention

[23] *I.e.*, with KCl the more dilute solution is the more positive but with NaCl it is the opposite.

[24] There is usually a difference between the end and the middle of a cell.

may be drawn to the fact that considerable experimental control is possible.[25]

For example, the current of injury can be made positive or negative or reversibly abolished. The order of apparent ionic mobilities in the protoplasm can be reversed (p. 220). The magnitude, duration, and threshold, as well as the shape of the action curve can be experimentally modified in a variety of ways (p. 226). The negative variation can be made to pass a dead region by means of a bridge (p. 223). Irritability can be abolished for weeks and restored at pleasure. Pacemakers are readily set up or suppressed (p. 229) and their frequencies can be regulated.

Mechanical disturbances traveling along the cell can be made to produce either positive (p. 229) or negative variations. Resistance and polarization can be greatly modified (7). In short, the whole range of phenomena can be experimentally controlled.

The continuance of such studies requires a knowledge of the properties of non-aqueous liquids, such as we find at the surfaces of the protoplasm. These layers are important not only in determining electrical phenomena but also in controlling the processes of absorption and excretion (66) (72). Since the physical chemistry of such substances is attracting more attention (60) (91) (92) we may hope that progress in this field will soon make it possible to attack many of the biological problems now awaiting solution.

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VITAMIN C, ASCORBIC ACID

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The isolation and identification of vitamin C has led to such an extensive literature since 1932 that the present review will be confined almost entirely to research papers which have appeared during the last four years. Excellent reviews of the earlier literature are readily available (1) (2) (3). Even with this limitation in scope, it has been necessary to further restrict references to the papers which have made outstanding contributions to current progress. The element of unfairness which is essentially involved in the omission of specific reference to valuable papers is greatly regretted, but the mass of papers dealing with the occurrence of the vitamin in foodstuffs, and the many suggestions of possible reactions, functions, formulas, etc., without adequate experimental support, has made many omissions necessary. The period covered by the present review has witnessed the identification of the vitamin, followed rapidly by establishment of its molecular structure, many exact measurements of its physico-chemical properties, synthesis in a number of research laboratories, commercial production of the natural and synthetic product, development of many methods of analysis, an extension in detailed knowledge of its occurrence in plant and animal tissues, a certain degree of correlation with specific enzyme systems, and a steady but slow advance in understanding its significance in relation to human health.

Identification and isolation. Laboratory experimentation in relation to vitamin C was largely made possible by the introduction of the guinea pig as an experimental animal by Holst and Frölich (4). Sustained investigations directed toward the isolation of the active substance were followed particularly by Zilva and associates (5), Bezssonoff and associates (6) and King and associates (7) during a period of several years. Each of these laboratories had devised varying methods of procedure, and had obtained strongly reducing, syrupy concentrates. Zilva believed that the strongly reducing substance present was a protective factor for the vitamin (8), and Bezssonoff interpreted his results as indicating that the vitamin was a dual or a tripartite factor consisting

of a phenol, a reducing sugar, and an organic acid (9). The rapidity of destruction of the vitamin under conditions of mild oxidation had been widely recognized. Tillmanns and associates (10) particularly pointed out the striking correlation between the antiscorbutic value of foods and their quantitative reduction of 2,6-dichlorophenolindolphenol, an oxidation-reduction indicator introduced into biological work by Clark and associates (11).

The first definite identification of the vitamin was reported by Waugh and King (12) (13), followed in a short time by independent confirmatory evidence from Svirbely and Szent-Gyorgyi (14) (15), and from Tillmanns and associates (16). Essentially these first papers by Waugh and King were based upon isolation of their first antiscorbutic crystals in August–September 1931, followed by finding the same degree of antiscorbutic activity after precipitation of the lead salt from alcohol (in which the free vitamin was very soluble), and again the same activity (8 weeks minimum protective level 0.5 mgm. per day) when the first crop of crystals had been recrystallized three times from different solvents. These crystals, obtained from lemons, corresponded in type, melting point, solubility, titration value against oxidizing and alkali reagents, rotation, electrical transference, and C-H combustion, with the "hexuronic acid" which Szent-Gyorgyi had isolated as a tissue respiratory factor from cabbage, oranges, and adrenal glands, at Cambridge University in 1928 (17). Svirbely (in Hungary on a post-doctorate Fellowship from the University of Pittsburgh) and Szent-Gyorgyi reported that the latter's preparation from adrenal glands was protective against scurvy during an 8 to 12 week period on a 1 mgm. level and cited the general correlation between the occurrence of the acid in oranges, lemons and cabbage and antiscorbutic activity.

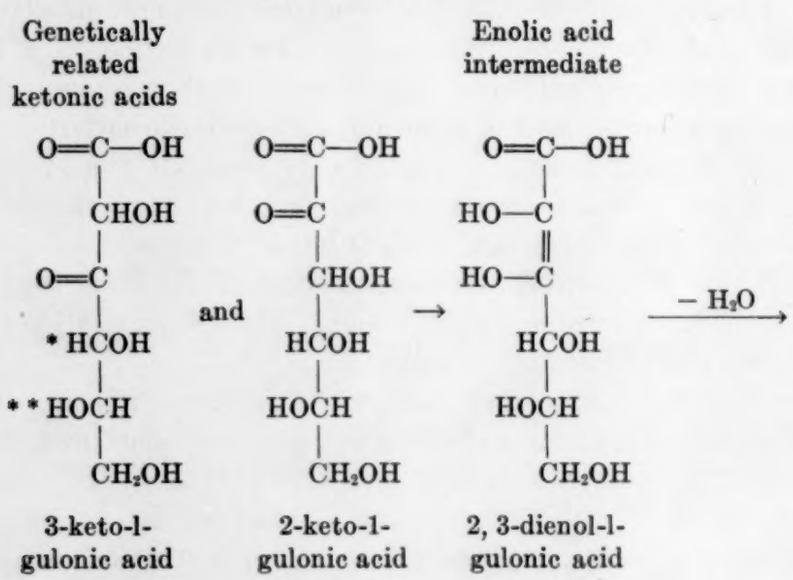
Rygh and associates had just previously reported the identity (by supposed isolation and synthesis) of vitamin C with methylnornarcotine (18), apparently as the result of *a*, their failure to recognize scurvy, and *b*, the addition of antiscorbutic food to some of the diets used. This report proved to be completely erroneous. There is no relation between the narcotine derivative and vitamin C.

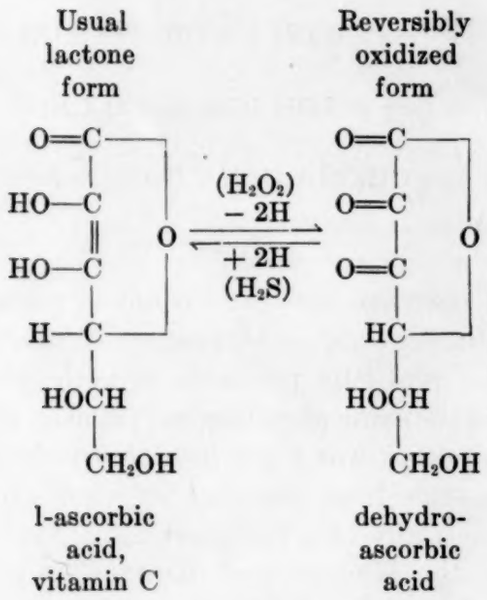
Within a short time, a large number of papers confirmed the true identity of the vitamin as a single substance, a six-carbon sugar-acid, as the name "hexuronic acid" indicated (19) (20) (21) (22). The chief argument raised by Zilva against the identity of the vitamin, based upon reducing capacity varying from the relative antiscorbutic activity of his laboratory preparations, was clarified experimentally by Till-

manns, Hirsch and Jackisch (20) in that the first oxidation product (unstable and reversible) remained effective antiscorbutically, as suggested by Waugh and King (13). Johnson (23), Zilva (24) and others have confirmed this relationship. The preparation of an acetone derivative by von Varga (25), which permitted independent purification and then recovery of the original vitamin (15) without loss of activity, was a very important step. There was no reasonable basis for questioning the identity of the vitamin, however, after it was shown that Kendall's (13) independent preparation, from adrenal glands and by an entirely different procedure, had an antiscorbutic value identical with the product obtained by Waugh and King. No other natural product has been found which can exert antiscorbutic activity in the guinea pig, monkey or human.

Synthesis and general chemical properties. In contrast with the time consuming problem of isolation, which was dependent upon animal assay for guidance, the course of molecular structure, synthesis, and commercial production was run through in a short period.

The first detailed reports of structure studies were from Haworth's laboratory (26), where a 10 gram sample of the "hexuronic acid" prepared by Szent-Gyorgyi had been sent for chemical study about a year before its significance as a nutritive factor had been discovered. Karrer's laboratory (27), H. von Euler's laboratory (28) and Micheel and Kraft (29) followed with a series of important studies leading to final acceptance of the following structural relationships:





* Essential configuration for the dextro-rotatory lactone.
** Essential configuration for the levo-series of sugars.

It will be noted that only C-atoms 4 and 5 must have a definite space position, or optical configuration. The closely related sugars have the following configurations:

CHO	CHO	CHO	CH_2OH	CH_2OH	CHO	CH_2OH
HOCH	HCOH	HCOH	HOCH	HOCH	HOCH	CO
HCOH	HCOH	HOCH	*or* HOCH	HOCH	HOCH	HOCH
HOCH	HOCH	HCOH	HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	HCOH	HOCH	HOCH	HOCH	HOCH
		CH_2OH	CHO	CH_2OH	CH_2OH	CH_2OH
l-xylose	l-lyxose	d-glucose		sorbitol	l-gulose	l-sorbose

Before the complete structure had been established, Reichstein and associates (30) accomplished the synthesis of an isomeric d-form, and a short time later the natural l-form, by the following series of reactions: xylose → xylosazone → xylosone → xylosone nitrile → 6-C acid, identical with the vitamin (but constitution uncertain). Haworth and associates suggested the correct formula (31) as one of a series of possible tautomeric isomers, and primarily proved that the formula now generally accepted is correct. In a detailed study of the l-xylosone series of reactions, the following steps were shown:

$$CH_2OH\cdot(CHOH)_3\cdot CHO \xrightarrow{\text{osazone}} CH_2OH(CHOH)_2\cdot CO\cdot CHO$$

$$\xrightarrow{\text{nitrile}} CH_2OH\ (CHOH)_2COCHOH\cdot CN$$

$$\xrightarrow{\text{hydrolysis}} CH_2OH\cdot(CHOH)_2CO\cdot CHOH\cdot COOH$$

$$\xrightarrow{\text{acid}} CH_2OH\cdot CHOH\cdot CH\cdot COH{=}COH\cdot CO \quad (\text{O bridge})$$

A further series of reactions was carried out in going from d-galactose through d-galacturonic acid → l-lyxose → l-lyxosone (l-xylosone) → ascorbic acid. The synthetic products in both laboratories had the same properties and the same physiological value as the natural product. Much of the organic work was made possible by the preparation of the vitamin on a large scale from paprikas by Szent-Gyorgyi, Banga and Svirbely (32). Practically identical methods of synthesis were worked out independently by Micheel and Kraft (33) and by White (in the author's laboratory), involving the following series of reactions:

$$\text{d-glucose} \xrightarrow{H_2} \text{sorbitol} \xrightarrow{\text{B. Xylinum}} \text{l-sorbose} \xrightarrow{C_6H_5NH\cdot NH_2} \text{l-sorbosone}$$

$$\xrightarrow{Br_2} \text{l-sorburonic (2-ketogulonic) acid} \xrightarrow{HCl} \text{l-ascorbic acid.}$$

A particularly successful method of synthesis was accomplished by Reichstein and Grüssner (34), which has been used in the commercial synthesis of the vitamin:

$$\text{d-glucose} \xrightarrow{H_2 \text{ and Pd}} \text{sorbitol} \xrightarrow{\text{Bact. xylinum}} \text{sorbose}$$

$$\xrightarrow{\text{acetone}} \text{diacetone sorbose} \xrightarrow{KMnO_4} \text{diacetone sorburonic acid} \xrightarrow{HCl}$$

$$\text{l-sorburonic (2-keto-1-gulonic) acid} \xrightarrow{HCl} \text{l-ascorbic acid}$$

(20–30 per cent yield from sorbose). Their later method for preparing the vitamin from d-galacturonic acid appears less practical (1935).

The name, ascorbic acid, was suggested by Haworth and Szent-Gyorgyi (35) before the structure was fully established, but when it had become probable that the vitamin did not possess a simple uronic acid structure characteristic of the regular 6-C series.

A number of structurally related compounds have been prepared (36), some of which possess a certain degree of antiscorbutic activity during short test periods, but no other substance has been found which has more than a fifth as high antiscorbutic activity as the true vitamin. The d-isomer is totally inactive (l-rhamnoascorbic acid = 1:5, d-araboascorbic acid = 1:20 and l-gluco-ascorbic acid = 1:40). In general chemical behavior, the other dienol lactones resemble ascorbic

acid very closely. From the work in Haworth's and Karrer's laboratories it appears that the acidic dissociation and the first mono-methyl substitution occur on the second enolic group (C-atom 3), the lactone configuration not being opened readily in neutral or acid solution. Some organic chemists are not entirely satisfied with the structural formula which is now assigned, however (37).

The oxidation-reduction potential (38), dissociation constants (39), absorption bands (40), optical dispersion (41), x-ray characteristics (42), and crystal forms (43) have been observed in a number of laboratories. Borsook (in press) has shown that the first oxidation product, dehydroascorbic acid, changes (rapidly in neutral or alkaline and slowly in acid solution) to another substance which is more strongly reducing and which cannot be reduced to the original vitamin either *in vitro* or *in vivo*.

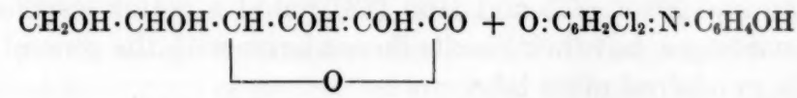
A brief summary of the properties of ascorbic acid would be: m. p. 192°, rotation + 24° in water (no mutarotation) and + 48° in alcohol, dissociation $pk_1 = 4.17$ and $pk_2 = 11.57$, absorption maximum 260 mμ in water and 263–5 in alcohol, rotatory dispersion in water $\alpha\lambda = 2.518/(\lambda^2 - 0.195) - 1.390/(\lambda^2 - 0.063)$, monoacetone ascorbic acid m. p. 220°, $E_{\frac{1}{6}}$ value for the oxidation-reduction potential at pH 4.0 and 35° = 0.166V.

Methods of analysis. The relatively close correlation between vitamin C biological assays and the titration values against 2,6-dichlorophenolindophenol shown by Tillmanns and associates (10) (16) (20), Harris and associates (44), and Bessey and King (45), together with the added confidence of dealing with a definite substance, has led to the widespread use of the indophenol reagent for vitamin measurement. Three hazards are particularly involved in titrations of this type, however, and due caution should be exercised in the interpretation of such data: *a*, other substances may be present which reduce the titration reagent; *b*, a portion of the vitamin may be present in the reversibly oxidized form, and *c*, substances may be present which interfere with the reaction of either the oxidizing or reducing agent. In only a few cases has there been evidence of serious error when the titrations were carried out rapidly in acid solution. Brain tissue (46), eye lens and aqueous humour (47), cancer tissue (48) and to a lesser extent, liver, apparently contain small amounts of unidentified non-vitamin reducing substances which react rapidly with the dye. Interference by glutathione, phenols, tannins and a number of other substances can be practically eliminated by titrating in strongly acidic solutions, with a

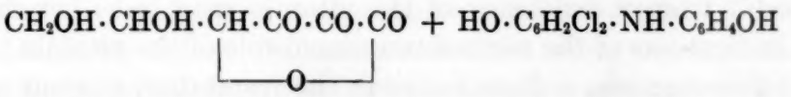
rapid end-point (about 5 seconds). Cysteine, H_2S, etc., must be removed or corrected for independently. Glucic acid (reductone) (49), reductic acid, and other sugar decomposition products may occur in heated (alkaline reaction) products. Beer and malt both appear to give very misleading values (50) (55). Apparently a fairly large proportion of the vitamin in blood (51) and other exposed tissues may be present as, or converted to, the reversibly oxidized form, requiring preliminary reduction by H_2S before titration. An element of uncertainty is introduced by the H_2S reduction, however (52). Other substances than dehydroascorbic acid may be reduced and included in the titration, and extra manipulations are required for removal of the gas. The use of mercuric acetate for removing interfering organic sulfur compounds and thiosulfates (53) may prove of value in special cases, but there is great danger in losing the vitamin in making precipitations with Pb or Hg salts.

The ferricyanide titration introduced by Tauber and Kleiner (54) has also met with general acceptance for titrating the vitamin. In view of the stability of the color and the simplicity of preparing the reagents, the method has certain advantages. The use of a purified specific enzyme (55) to oxidize the vitamin may provide a useful technique in restricted types of analyses. The color reagent ($MoO_3P_2O_5(WO_3)_{17}$-$(H_2O)_{24}$) introduced by Bezssonoff (56) serves for estimating the vitamin in certain cases, but the reagent is apparently not as specific as the others. Roe (57) has introduced a method based primarily upon decomposition of the ascorbic acid into furfural, and development of the usual color with aniline. Differentiation from common uronic acids and pentoses is based upon the ease of destroying the furfural-yielding capacity of ascorbic acid by mild oxidation. Sullivan and Hess (58) have introduced a special procedure whereby ascorbic acid, glutathione, cystine, and cysteine can be determined independently. This method appears to be especially valuable in analyzing such tissues as eye lens and cancer tissue. Melville and Richardson (59) have reported the use of an oxazine dye, prune, for direct titration, but in the author's laboratory the reagent was not of practical value. Bachstez and Cavallini (60) have suggested the use of $UO_2(OAc)_2$ to differentiate ascorbic acid from isoascorbic acid. Szent-Gyorgyi (61) has suggested the use of a color reaction with $FeSO_4$, exposed to air. Martini and Bonsignore (62) have reported that reduction of methylene blue is more specific than indophenol for ascorbic acid (not in agreement with results in the author's laboratory). Micro methods of titration with the indophenol

dye have been described by Birch, Harris and Ray (63) and Glick (64). The latter has extended the Bessey and King method to such a micro scale (Linderstrom-Lang technique) that microtome slices can be accurately analyzed and the vitamin content of a single cell estimated with fair accuracy. This technique is particularly valuable in the study of vitamin C distribution in glandular tissues. Measurement of the intensity of absorption bands in tissue extracts has also been satisfactorily adapted to general laboratory practice by Robertson (40) (65) (Hilger). Fujita and Iwatake (66) have demonstrated the advantage of two per cent metaphosphoric acid for extraction of the vitamin, replacing trichloroacetic acid, which causes a slow oxidation. They also found sodium tungstate suitable as an oxidizing agent, giving a satisfactory blue color reaction. Eckelen (67) found the method unsatisfactory, however. King and Musulin (68) have modified the indophenol technique to include the use of metaphosphoric acid. The latter exerts a marked stabilizing action on the vitamin in solution, but it is not a very satisfactory deproteinizing agent. The reaction of the dye with the vitamin may be indicated as follows:



Ascorbic acid — Indophenol dye, red in acid, blue in alkaline solution



Dehydroascorbic acid, or 2,3-diketogulonic acid lactone — Phenolic leucobase, colorless

From a purely theoretical basis, it would appear that other oxidizing agents should be more specific in titrating the vitamin, but the rate of reaction in acid solution is so satisfactory with this dye that no other reagent has been so widely adopted.

Biological methods of assay. Chemical methods have rapidly replaced biological assays for many types of investigation, but there has been a continued need for animal assays to supplement chemical analyses, particularly in view of the risk of misinterpreting chemical tests.

The Sherman, La Mer and Campbell technique (69) has continued to be most generally used for assay purposes, although many investigators have shortened the protective period to 6 or 8 weeks and have added yeast and cod liver oil to the diet. For more rapid assay work, the

curative test, as outlined by Harris and Ray (22) has proved very useful. Less material is required and there is a considerable economy of time and cost with the curative test. The tooth cross-section method of Höjer (1) continues to be used fairly widely, but has the disadvantage of requiring special equipment and techniques seldom at the disposal of a chemical laboratory. The protective level for normal teeth is practically twice as high as the level which is protective against general scurvy. Dann and Cowgill (70) found that the vitamin C requirement of guinea pigs from 120 to 1040 grams in weight was approximately 1 cc. of lemon juice per 100 grams body weight, irrespective of age, as measured by the tooth-section method. They suggest that there is not an additional tissue requirement for vitamin C coincident with a higher metabolic rate in young animals, but this conclusion is not in apparent harmony with the finding of a higher content of the vitamin in young animals, which are capable of synthesizing the vitamin (45).

In administering the pure vitamin to guinea pigs, Gorski and King (71) found little difference in protective or curative effect when the solutions were fed, injected subcutaneously, or injected intraperitoneally. Grollman and Firor (72) and Hou (73) found a better response from injection dosages, but their results do not agree with the general experience of a number of other laboratories.

Physiological functions. a. In relation to specific enzymes. The macroscopic (1) and microscopic (74) (75) changes in animal tissues which result from a deficiency of the vitamin may be either direct or indirect indications of the normal functional rôle of the vitamin in those tissues. For example, a disturbance in the respiratory enzyme systems in the pituitary gland or the corpus luteum, resulting from a slight vitamin deficiency, might result in physiological disturbances in the other tissues of an animal which would be difficult to correlate directly with the primary nutritional disturbance. From a chemical point of view such complex problems can be studied to best advantage in terms of specific molecular reactions, such as the relation of a single purified substrate to a single purified enzyme. Then the influence of other reactants can be correlated and interpreted in terms of the primary reaction. In the case of vitamin C this phase of study is well under way—with the advantage of a 4-year start, previous to the identification of the vitamin.

The "hexuronic acid oxidase" in the adrenal cortex and cabbage leaves, described by Szent-Gyorgyi (17) (76) and Kendall (77), has been accepted as evidence of the fundamental importance of ascorbic acid in tissue respiration, the vitamin playing the rôle of a hydrogen-transport

agent between organic metabolites, and indirectly, molecular oxygen. Tauber and Kleiner have succeeded in preparing a second oxidative enzyme from hubbard squash which is apparently specific for the vitamin (55) and which causes it to react directly with free oxygen. It will be interesting to find whether this enzyme is also present in animal tissues.

Definite interpretation of apparent relationships between the vitamin and enzyme systems acting on other substrates is particularly hazardous because of the possible influence of unrecognized substances. The strong reducing action of ascorbic acid may cause it to alter such reversible systems as: $Cu^{++} \rightleftharpoons Cu^{+}$, $Fe^{+++} \rightleftharpoons Fe^{++}$, S—S $\rightleftharpoons$ 2SH, $Mn^{+++} \rightleftharpoons Mn^{++}$, and C=O $\rightleftharpoons$ C—OH, thus exerting an indirect effect in vitro, which is not specifically or essentially related to the vitamin in vivo. This danger is particularly important because of the general use of enzyme systems which are not highly purified, but very sensitive to such changes as indicated above (78) (79).

A number of papers have discussed the relation of ascorbic acid to the enzyme systems in cancer cells (48) (80) (81) (82). These have centered around the activation of arginase, β-amylase and cathepsin, and the auxiliary activating effects when a Cu^{++} of Fe^{++} complex is formed (83). A marked activation of papain by the Fe^{++} complex has also been reported. Purr (84) has emphasized the significance of the S—S groups in accompanying proteins or glutathione for the activation by ascorbic acid—Fe^{++}. Inactivation or inhibition has been reported for: ascorbic acid—Cu^{++} on urease (85), ascorbic acid alone on tyronsinase (including the dopa reaction) (86), dehydroascorbic acid on α-plant amylase (87), ascorbic acid (without Fe) on papain (83), ascorbic acid on catalase (83), and ascorbic acid on phosphatase (88). Nearly all investigators are agreed that small amounts of Cu catalyse oxygen uptake by the vitamin alone and that Cu tends to inhibit the activating action of ascorbic acid or ascorbic acid—Fe^{++} on the proteolytic enzymes. The increased oxidation of fatty acids in the presence of ascorbic acid (89) was not correlated with specific enzymes. Maschmann (90) believes that the effect of added Fe is indirect, in removing an inhibitor substance, and that if the enzymes papain or bromelin are purified from this substance, either ascorbic acid alone or Fe is without effect, or may even serve as an inhibitor.

The reported dehydration and formation of NH_3 from leucine, by dehydroascorbic acid (91) is of particular interest in suggesting a new rôle for the vitamin in intermediate metabolism.

Pfankuch (92) presents evidence for the presence of an enzyme in potato juice which catalyzes the reduction of dehydroascorbic acid by cysteine.

Physiological functions. b. Distribution and function in plants. The two oxidative enzymes specifically related to the vitamin by Szent-Gyorgyi and Tauber and Kleiner are probably distributed very widely in the active tissues of the higher plants. The vitamin is known to be formed rapidly in sprouting seeds, becoming evident within a few hours (1) (92), and it apparently reaches a high concentration in all rapidly growing stem or root tips, green leaves, seeds and pods. Practically all fresh fruits and tubers contain significant amounts of the vitamin. Accordingly it is reasonable to assume that the vitamin plays an active functional rôle in all these various tissues, simply varying in ascendency and concentration with different types. Its relatively higher concentration in the tissues where metabolism is highest is further evidence of an essential metabolic rôle. However, the high concentration in citrus fruits, paprikas, tomatoes, rose hips, etc., constitutes something of an exception to its correlation with a high degree of growth or metabolism. In all plant tissues studied, where secondary oxidation had been avoided, the ascorbic acid has been present almost entirely in the reduced form. v. Euler and Klussmann (94) have reported a possible exception in sprouting seeds, where they found a titration value lower than the anti-scorbutic value, but Johnson (95) has reported finding just the reverse.

The number of examples where there is a coincident high concentration of vitamin C and green, yellow and red pigments (45) (96) affords a basis for interesting speculation and future experimentation, but so little is definitely established concerning the rôle of either the pigments or the vitamin that one can scarcely do more than note the association in occurrence at the present time. Many plant physiologists are of the opinion that the associated pigments are participants in cellular respiration, and the parallel occurrence of vitamin C in high concentration adds some weight to this point of view.

The close correlation between photosynthetic activity and vitamin concentration points toward an essential relationship, but since the vitamin precedes the appearance of chlorophyll and the carotenoid pigments in the growing plant (96), it is clear that vitamin synthesis is only indirectly dependent upon photosynthesis. There is probably an immediate dependence in the other direction, in which ascorbic acid enters into the series of reactions comprising photosynthesis. Further work with the lower plant forms, and micro investigations of leaf-cell

layers should prove very fruitful. It would be interesting to know the stage of development in plants where vitamin C appears, since the lower forms such as bacteria and yeast apparently do not contain it. Norris (97) has reported a fairly high concentration in some of the algae, and low values for others. Chen (98) found appreciable amounts in edible fungi.

Important and extensive surveys have been made concerning the vitamin C value of foodstuffs during the past few years, particularly in Russia (99), Germany (20), India (100), China (101), and the United States (45) (102).

The precursor of vitamin C in plant tissues has not been definitely established. Ray has found mannose superior to the other hexoses for vitamin synthesis in pea seedlings (103), but much further work is necessary to clarify the problem (104). A brief study of the reduction of nitrates and nitrites was reported by Karrer and Bendos (105).

Physiological functions. c. Distribution and function in animal tissues. From the available data it seems reasonable to conclude that ascorbic acid is present in practically all tissues of the higher animals. In general, it is highest in the glandular tissue and lowest in muscle tissue and stored fat. Tissues such as the heart, kidney, and lungs are intermediate. The adrenal, pituitary, corpus luteum and young thymus are highest of the glandular tissues, with pancreas, liver, spleen, testes, ovaries, brain, thyroid, sub-maxillaries and intestinal walls showing concentrations much higher than muscle tissue. It is evident that the concentration of vitamins A, B and G, in glandular tissue coincides with the distribution of C only in a very general way.

The two specific rôles for vitamin C in animal tissues which appear to be clearly established are: *a*, a respiratory function (cited above) in serving as a hydrogen-transport agent between unidentified metabolytes and other carriers or molecular oxygen, by way of two or more oxidase enzyme systems; and *b*, regulation of the colloidal condition of intercellular substances as shown by Wolbach and associates (74). Most of the gross effects of severe scurvy can be reasonably well explained on the basis of these two functions. As indicated in the discussion of enzymes, and later in this section, however, it appears likely that the vitamin effects, directly and indirectly, many additional types of chemical change.

The high concentration of ascorbic acid in glandular tissues indicates a degree of correlation with the general rate of metabolism analogous to the relationship in plants. This may be in part a direct respiratory

influence, and in part an acceleration of the activity of other enzymes particularly the arginases, proteases and amylases. The total oxygen uptake of the body is decreased with the onset of scurvy (106) and tissue slices from scorbutic animals show an increased oxygen uptake upon addition of the vitamin *in vitro* (107) (89). The relation to intercellular substance provides an explanation for the gross tissue changes that are primarily characteristic of scurvy, e.g., tooth changes, bone changes, and hemorrhages from weakened capillaries. From a chemical point of view, however, the basis for this function of controlling the intercellular substance is not clear.

It is interesting to note that the relative distribution of the vitamin is nearly the same in the tissues of animals which synthesize the vitamin, such as rats and chickens, and in the animals which require it in their food supply, such as the guinea pig and human, and that the former contain much more than the latter when on conventional diets (45) (108). Human tissues correspond with those of the guinea pig (109).

When the guinea pig (110) or human (111) is on a vitamin C-free diet, excretion in the urine continues and the body tissues are markedly depleted before there is any external indication of scurvy. The quantity destroyed by metabolic processes can only be approximated from data available.

The vitamin C content of cow's milk has been studied rather extensively in relation to feeding practice and methods of merchandising (1) (112). Confirming earlier work, it is evident that the antiscorbutic value of cow's milk is maintained at a normal level under common feeding conditions, and that the degree of destruction by pasteurization may be small or great, depending upon the degree of exposure to air and copper. During the past year it has been shown that mother's milk is normally about 5 times richer than cow's or goat's milk in vitamin C content (113) but when the diet is inadequate, the value may drop to subnormal levels.

The claim which has been recently made, that developing human and guinea pig embryo tissue (114) and new born infants (115) can synthesize their own supply of vitamin C is probably erroneous. The evidence from which the suggestion arose was inadequate, and it is not in agreement with the data obtained in other laboratories. Guinea pigs may develop scurvy or at least show vitamin C deficiency during gestation (116), mothers may become depleted during pregnancy to such a degree that they are too deficient in vitamin reserves for normal lactation (113), and very young infants have been found at autopsy to be deficient in

vitamin C reserves (109). If such a capacity exists, it clearly cannot be relied upon for protection of either the maternal organism or the offspring.

The manner of synthesis of ascorbic acid in animals which do not require a dietary supply has not been clarified. It is formed from some constituent of egg white in the chick embryo very soon after growth begins (117). Neither the site of synthesis nor the substance from which it is formed has been identified. Liver perfusion experiments have provided an indication of its synthesis, but the original substance and the resulting products were not definitely identified. In other experiments rat liver, kidney and spleen converted only mannose to a reducing substance (118). Ray also reported that mannose was more effective than other hexoses for the synthesis of ascorbic acid in sprouting seeds (119). Mosonyi has suggested $CH_3 \cdot CO \cdot CHOH \cdot CH_2 \cdot COCH_3$ (Henzel's ketol) as an intermediate used in the synthesis of ascorbic acid (120) and has reported a rise in the reducing value of rat adrenals after its administration.

Recent studies of the changes in composition of the blood and various tissues accompanying the onset of scurvy have not revealed striking changes. There is an increased acidity (and fatty acids) (121) and a decreased hemoglobin (122) with a delayed clotting time (123).

The loss of normal resistance against infections caused by a deficiency of vitamin C has been further established (124). It has also been shown that guinea pigs depleted of their vitamin C reserves were less resistant to bacterial toxins (125) as measured by poorer survival and a greater degree of necrosis (126). The lowered resistance could be shown clearly in the depletion zone before there was any external evidence of scurvy. Of particular interest in this work was the finding that repeated sublethal injections of toxin regularly produced in the subnormal animals a diffuse hyperplasia of the arterial wall. Although the development of this type of arteriosclerosis was not identical in all details with the histological picture seen in human arteries, the resemblance was close enough to appear significant. In addition, the adrenals, liver and islet tissue of the pancreas were injured, and there was an accompanying lowered sugar tolerance (126). The physiological basis for a lowered sugar tolerance has not yet been clearly defined. Similar results with rabbits (not subject to scurvy) were thought to be due primarily to liver injury (127). As a result of the toxin injections, a rapid and marked depletion of the vitamin from the glandular tissue was observed (128). Euler and Klussmann observed a 50 per cent

depletion of ascorbic acid from the adrenals of guinea pigs following methylene blue (2 mgm. per kgm. body weight) injections (129). Phosphorus poisoning produced no change in titration values for rats, however (130). In comparing high and low vitamin C levels, Mitolo (131) found that the latter were more sensitive to both metal and metalloid poisoning. Destruction of the vitamin *in vitro* by intestinal bacteria was reported by Stepp and Schroeder (132). The lesions produced in mildly scorbutic guinea pigs by streptococci infections, resembling the lesions of rheumatic fever (133), were not considered significant in clinical work (134). Phillips, Stare and Elvehjem (135) reported a decreased indophenol oxidase activity of the liver, coincident with either scurvy or fluorosis. A decreased ascorbic acid value was observed for the anterior pituitary, but not for the liver, kidney or pancreas, as a result of fluoride poisoning of guinea pigs. The liver was not changed in oxygen uptake by either scurvy or fluoride, but a lowered oxygen uptake by the adrenal was observed. In fluoride-fed rats there was a rise in vitamin C content of the anterior hypophysis and the adrenals, but no rise was observed in the vitamin C content of the liver or kidneys (136). Only slight increases were observed in the titration value of cow's tissues as a result of fluorosis (137).

The fact that silver nitrate staining is not reliable, qualitatively or quantitatively as an index of vitamin C in tissues, may explain the difference in results obtained in another laboratory (138). Staining of the adrenal cortex provides a fair approximation of the vitamin content, however.

Bourne (139) observed granules, indicated by silver deposition and interpreted as consisting of vitamin C, surrounding the lipid globules in the adrenal cortex. Very little evidence is available concerning the distribution or state of the vitamin in individual cells, however. There is some evidence of a loose type of combination with —SH or other protective groups (140).

Hartman and associates (141) reported a moderate extension of survival period when guinea pigs placed on a vitamin C-free diet were given cortical hormone extracts, but Svirbely (142) and Grollman (143) did not find such a relationship. The function of ascorbic acid in the adrenal appears to be general rather than specific, nor is this gland the sole point of ascorbic acid synthesis (144) in non-scorbutic animals.

The intimate relation of faulty tooth development to low levels of vitamin C intake has been extended, particularly in the zone of nutrition where scurvy is not evident (145). Latent scurvy may also induce

characteristic peptic ulcers in guinea pigs (146). The disturbances in blood calcium, sodium and potassium coincident with scurvy are of a relatively low order (147). Fish and marine invertebrates have a distribution of vitamin C in their tissues much like that found in the higher animals (148), but the presence or absence of ascorbic acid in tissues in relation to their respiratory systems or biological classification has not been studied extensively.

Studies in relation to the general food supply. The citrus fruits and tomatoes continue to occupy first place as antiscorbutic foods, but there are many reasons for conserving the vitamin C value of the general food supply, rather than depending entirely upon a special source. The fact that destruction of the vitamin is generally parallel with injury to other food qualities such as flavor, color and other vitamins, adds importance to the problem of maintaining antiscorbutic activity.

A number of studies of the effects of drying (149), canning (150), cooking (151), freezing (152), and storage (153) have been made. The results have been in essential agreement with earlier work, indicating the unfavorable influence of *a*, contact with heavy metals, especially copper; *b*, exposure to air; *c*, high temperatures; *d*, disruption of cell structures; *e*, alkalinity, and *f*, exposure to light. Of particular interest was the report by Nelson and Mottern (154) that lead arsenate sprays caused a lower vitamin C value in oranges without evidence of an increase in lead or arsenic in the fruit. The studies by Morgan, Field and Nichols (151) showed that sulphur dioxide aids in preserving the vitamin content of dried fruits. The influence of light in accelerating the oxidation of ascorbic acid in milk has been shown to be important (155). The effect of freezing, with a certain degree of disorganization of the cellular structures in foods, calls for special care to avoid oxidation after thawing (156). It will be interesting to watch the rate of progress in introducing synthetic or natural vitamin C into special foods but there has been practically no advance in that direction up to the present time.

Many of the large canning companies now market foods with a vitamin content which approximates that of carefully cooked fresh products. The improved methods in canning and refrigeration should do much to off-set the handicap of an urban population in obtaining common foods of high antiscorbutic value.

Vitamin C in relation to human health. Clinical scurvy is still observed occasionally in most parts of the world, but the incidence is not large. Among the more significant reports are those of Dalldorf (157), Göthlin (158), Ohnell (159), Nordenmark (160), Schultzer (161), Dry (162),

Stocking (163), Gedda (164), and Ross (165). Repeated surveys in Scandinavia have shown that about 20 per cent of the children and an appreciable proportion of the adult population have a vitamin C intake during the winter and spring months which is low enough to cause a distinct weakening of the blood capillaries. Dry found 60 per cent of the laborers (10,000) in an African locality (Rhodesia) afflicted with mild or severe scurvy. Dalldorf reported that weakened capillaries could be demonstrated in 35 to 66 per cent of the children received from poor homes into the Grasslands Hospital, New York. The greater number of these cases responded immediately when antiscorbutic foods were given, showing that the disorder was primarily due to a specific form of malnutrition. Hanke and associates have presented an impressive record (145) of their studies in relation to tooth and gum disorders. In determining the vitamin C content of human tissues from general hospital autopsies in Pittsburgh (109) it was found that about 20 per cent of the patients had been depleted, either by dietary practice or pathological effects, to levels which were clearly subnormal.

The fact that there is a wide zone of vitamin deficiency between scurvy and optimum health is of more interest in relation to human health than the problem of clinical scurvy. Quantitative studies in this zone of malnutrition are no longer impossible. The newer methods of diagnosing mild types of vitamin deficiency, by means of measuring capillary strength or by direct titration of the vitamin in blood (51) (166), spinal fluid (167), and urine (168), provide a basis for better evaluation of vitamin C levels than has been possible in the past. The rise in indophenol titration value of urine after a standard dosage of vitamin C provides a simple, rapid and direct means of evaluating the true condition of the body tissues in terms of the vitamin reserve.

The human requirement per day appears to range from about 25 mgm. in infancy to about 40 mgm. in adults. Quantities above the required amount are eliminated rapidly by the kidneys without damage. An estimated allowance in dietary sources should be well above the minimum requirement because of individual variations and because of the risk of loss before food is eaten. There is no evidence of serious loss during the process of assimilation from the digestive tract. It is also very probable that pathological conditions, particularly infections and nephritic disorders, may be accompanied by excessive losses of vitamin C from the body tissues (128) (169).

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THE ECONOMY OF MUSCULAR EXERCISE

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Muscular exercise has long been studied with enthusiasm by physiologists. Like cosmic rays and solar eclipses this subject gives much satisfaction to the adventurous investigator. The fields of sport and of war, the factory and the farm, the desert, the jungle and the mountains offer tempting problems. Even to those who prefer the circumscription of laboratory walls an unlimited number of problems is afforded by the complex organization of the human machine. It is not surprising, therefore, that interest in the physiology of muscular activity is world-wide.

It is hardly necessary to recall such familiar names as those of Benedict and Carpenter in Boston, Atzler in Dortmund, Krogh, Lindhard and Christensen in Copenhagen, Liljestrand in Stockholm, Cathcart in Glasgow, and Hill in London. Recently an institute for study of man has been founded by Laugier in Paris, another by Brouha in Belgium, a third by Teruoka in Japan, and a fourth "The Institute of Human Relations" at Yale.

Numerous physiological investigations of industrial situations have been carried on by Soviet laboratories in recent years. Typical of these is a study by Krawtschinsky of "acid-base equilibrium" of foundry workers (1). He found that venous blood usually underwent a slight decrease in carbon dioxide content and an increase in blood lactate during the course of a day's work. In another instance Brandis and Borschtschewski (2) made an analysis of brick-laying with measurements of physiological functions in relation to the output of the worker, and the kinematics of the process. They express the Soviet point of view in such investigations as follows: *"Das ganze System der functionell zergliederten Organization der Maurerarbeit . . . bedeutet eine mächtige Förderung, die die Arbeitsressourcen der betreffenden Arbeitskategorie maximal mobilisiert und von dem gesamten Rätebund in Weitesten Umfang übernommen werden musz."*

It is interesting to find that a point of view similar to that of the Soviets is held by German and English investigators. Thus in Atzler's institute much attention has been paid to the economy of the worker in various industries. Baader and Lehmann (3) have measured the energy requirement for brick-laying with three standard sizes of bricks and Wenzig (4) has made a study of shovelling with particular reference to load, height and dimensions of the shovel. Correspondingly, in England Bedale (5) of the Industrial Fatigue Research Board has made a *Comparison of the energy expenditure of a woman carrying loads in eight different positions.*

In this brief review many aspects of the subject will be touched on but lightly if at all. The reader interested in comprehensive discussions is referred to the work of Schneider (6) and of Gould and Dye (7) published in the United States; to Bainbridge, recently revised by Bock and Dill (8) and to Atzler (9). Among recent reviews are those of Hansen (10), Herxheimer (11), Hill (12) and Steinhaus (13). Records of original investigations are widely scattered in the literature—physiological, biochemical and medical, although two journals restricted to the field and of international scope are available. *Arbeitsphysiologie*, edited by Atzler, was established in 1928 and is now in its seventh volume. *Le Travail Humain*, which owes its inception to Laugier, is in its third year. These two journals have on their editorial boards representatives of many nations.

The title of this paper implies that muscular exercise depends on an efficient organization of bodily functions. Particular attention will be paid to the mobilization of bodily resources for various types of muscular activity, prolonged or short in duration, moderate or severe in intensity. When work is over, the original state must be restored; the energy-yielding processes in work are dependent upon the constructive processes of recovery.[1]

Moderate activity. Examples of this type of activity are on every side. In the modern factory while all tasks demand the expenditure of some energy few workers experience any considerable drain on energy reserves. Yet "fatigue" is a familiar term in the vocabulary of the personnel director and deserves some attention.

The usual methods of approach reveal that the increase in oxygen consumption is relatively small, the heart rate is but slightly elevated and changes in the properties of the blood are for the most part negligible. Since fuel is rendered available by digestive processes as work proceeds, little call is made on energy reserves. When the day's work is done, paradoxically, the expenditure of energy at a fast rate is apt to begin. The day in the factory frequently is followed by work in the garden, by strenuous games or by dancing. The policy in many industries of encouraging sports among their workmen would receive little support if the day's work produced physical exhaustion.

[1] Physiological measurements show that the athlete after maximal exertion lasting 10 seconds may not be back to normal in an hour; the observations of the trainer indicate that the processes of recovery are not complete in 24 hours. Probably no sprinter can run 100 yards in his record time daily; perhaps, not even weekly.

Classical methods for studying physical exercise have not been particularly fruitful in this field. The fatigue of the factory worker cannot be defined in terms of lactic acid, alkaline reserve, hydrogen ion concentration, hemoglobin or "toxin." The problem is too subtle to yield to the physiologist's measurements of pulse rate, blood pressure, respiration and circulation.[2] Finally, the sensory tests used by psychologists do not give complete satisfaction.

Recently a solution for the problem has been offered. The formula, familiar in law and politics but less well-known to scientists, consists of a change in name. It is suggested that the phenomenon formerly called fatigue is better described as boredom. The history of this interesting development is given by Mayo (15). Cathcart in 1928 (16) had written that of the various indirect tests devised to measure fatigue, "*probably, on the whole, the most reliable is that which measures performance or rate of output,*" the implication being that fatigue decreases output. A year later Wyatt and Fraser (17) of the Industrial Fatigue Research Board concluded that "*boredom causes a reduced rate of working which is particularly noticeable about the middle of the spell.*" The following year the Industrial Fatigue Research Board, founded in 1917 to study fatigue among munition workers, changed its name to Industrial Health Research Board. It is hardly necessary to point out that such a solution is pseudo rather than real. In fairness it must be added that the authorities of the Research Board presumably replaced the word "*Fatigue*" because their interests had shifted from those phenomena loosely described by that word to other subjects.

Research of merit usually calls for precise measurements. When the investigation has a subject so variable and undependable as man, the pains taken by the observer may be no more important than the number of observations. This principle has no better exemplification than in the work of Mayo's group in coöperation with the research staff of the Western Electric plant at Chicago (15). Over a period of several years a test group of five workers has labored under known conditions. Their job of assembling relays lends itself admirably to accurate measurement of rate of output. Suitable recording devices have yielded a complete record of the time required for every relay assembly by each operative in the experimental room. From these observations Whitehead (18) has calculated the rate of output of each operative per hour, per day and per week and has sought the explanation of the variations observed.

[2] For those interested in the results of such measurements reference can be made to Lovekin (14).

One of the outstanding features of his analysis "*is the comparative absence of the influence of the daily cycle on output rate. Perhaps there is a slight warming-up period at the beginning of the day; it is very slight. The most noticeable feature is a poor output for the first half of the afternoon with complete recovery after the 2:30 rest pause.*"

The common conception of factory labor is in conflict with these observations. According to this concept the workman begins his job in the morning with fatigue at a minimum and energy at a maximum. As the day passes there is continuous increase in fatigue while energy reserves are being utilized. Output is influenced unfavorably by increasing fatigue, but early in the day output may be kept up by the counterbalancing influence of warming-up. This theory implies that output rather than remaining constant should fall off as the day wanes. Such a concept of mounting fatigue could hardly be held by one who has experienced the pleasure of a full day's moderate work, of the hearty meals which accompany it and of the sound sleep which follows it. Fatigue and boredom are not concomitant with muscular activity; many, if not most, people outside the tropics enjoy work and dislike idleness.[3]

The Hawthorne experiment has taken into consideration the influence of environmental conditions on performance. The early experiments on the influence of illumination are not fully published but reference to them has been made by Mayo (15). He states that, "*the inquiry involved in one phase the segregation of two groups of workers, engaged upon the same task, in two rooms equally illuminated. The experimental diminution of the lighting, in ordered quantities. in one room only, gave no sufficiently significant difference, expressed in terms of measured output, as compared with the other still fully illuminated room. Somehow or other that complex of mutually dependent factors, the human organism, shifted its equilibrium and unintentionally defeated the purpose of the experiment.*" Some years later data accumulated over the five-year period included enough hot and sultry periods to enable Whitehead (18) to assess the dependence of output on weather. Again the answer is straightforward: within wide limits there is no significant dependence!

For the performance of a factory workman to be relatively independent of weather is in conflict with established ideas. An opposite viewpoint is found in Huntington's *Civilization and Climate* (19).

[3] Cathcart (16), on the other hand, refers to a German inquiry which indicates that only a small percentage of three groups of industrial workmen were interested in their job.

The output of Connecticut factory workers was at a maximum when the mean external temperature was 15°C. It is not our province to attempt a reconciliation of these divergent findings, although it is pertinent to inquire by what mechanism low external temperature influences work in a heated factory.[4] It is relevant to call attention to the well-authenti-

TABLE 1

Records of Western Electric experiments

PERIOD	DURATION	CONDITIONS	AVERAGE OUTPUT THROUGHOUT PERIOD
	weeks		*relays per hour*
I	2	Factory routine	49.8
II	5	Factory routine in test room	49.2
III	8	More favorable group rate of pay	51.0
IV	5	Two five-minute rest pauses	52.4
V	4	Two ten-minute rest pauses	55.4
VI	4	Six five-minute rest pauses	55.5
VII	11	Morning rest pause of fifteen minutes with lunch provided; afternoon pause of ten minutes	55.7
VIII	7	Like VII with working day shortened one-half hour	62.0
IX	4	Like VII with working day shortened one hour	64.0
X	9	Like VII	61.8
XI	12	Like VII with five-day week; summer time	62.9
XII	12	Like III	60.7
XIII	31	Like VII with employees supplying lunch	66.4
XIV	9	Like XI	63.2
XV	31	Like XIII	66.2

cated high work output of Maine lumbermen (20) in winter and to Arctic or Antarctic exploits such as the march of Amundsen and his party to the South Pole and return (21).

While the Hawthorne inquiry has revealed unexpected constancy of

[4] Cathcart (16) gives in his figure 5 a chart which shows a 10 per cent decrease in output of tin plate workmen as the mean temperature rises from 40 to 62°. Curiously enough, in figure 1, picturing the effect of an 8-hour day on output of steel workers, the curve increased irregularly 20 per cent over a period of 12 months, reaching its maximum in July, but then descended to another minimum the following spring.

performance throughout the day whatever the environment, long-range variations of the first order were found. Some of the more salient data of the five-year study, assembled by Whitehead[5], are found in table 1. It will be noted that a given set of conditions was maintained for some weeks or months and that late in the study an attempt was made to restore conditions of earlier periods. While output varied somewhat in relation to length of working day and rest pauses, the most significant finding is the distinct trend upwards in output throughout the five years despite the attempt to control conditions. The direct explanation of this result is largely sociological and hence outside the bounds of this paper. Apparently the most important contribution the physiologist can make is towards the solution of such problems as the hazards of industry, the health and nutrition of the worker and recreational programs. Their solution is relevant since the Hawthorne study seems to prove that with ideal social conditions outside as well as inside the factory the day's work will be carried on happily and at a uniform rate: by definition therefore with neither fatigue nor boredom.

Hard work. The capacity of the individual for supplying oxygen to his tissues has a definite limit. For the athlete this may be as high as twenty times the basal metabolic rate (BMR) but for the ordinary person the ratio is nearer 10. It does not follow that work can be long continued at the maximal capacity of the circulatory and respiratory systems. A much lower limit must be set for the rate of work which can be maintained for eight hours and from which recovery is complete in sixteen hours. We venture to say that for the man doing hard work a mean metabolic rate of about eight times the BMR is as much as can be maintained for eight hours. If the term *moderate work* is confined to activity which is less than three times, and *maximal work* to activity more than eight times the basal rate, we have arbitrary but useful lines of demarcation for *hard work*. Included in this area lie most of the manual jobs in heavy industries, building trades, mining, agriculture and war. Figure 1 has been prepared to show the relation between intensity of work and total caloric production for small, average and large men. Within the limits set, the old records of Maine lumbermen (19) with their production of 8,000 C. are off the scale. It is possible that the ratio Work MR/BMR is greater than 8 in their case. However, the length of the working day exceeded 8 hours and this may account for most of the difference.

There is unquestionably a critical level of activity below which

[5] Personal communication.

absorption of food proceeds normally and above which digestion, absorption and excretion are disturbed. It is clear that moderate work is below the critical level and maximal work above it. The introduction of rest pauses has been supported by the argument that digestion is thus promoted. In a recent study of a man engaged in hard work for eight hours daily, Engelman (22) found that beginning work imme-

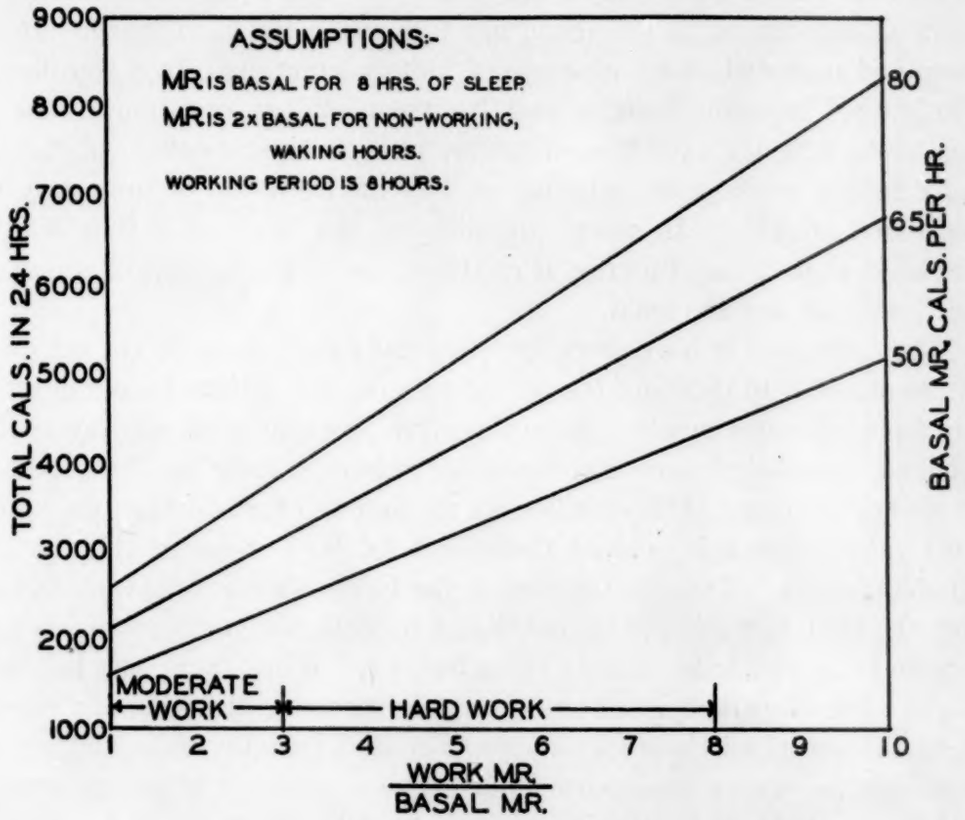

Fig. 1. The ratio of work metabolism to rest metabolism $\left(\frac{\text{Work MR}}{\text{Rest MR}}\right)$ in relation to total caloric production. The three lines correspond to individuals with BMR values of 50, 65 and 80 cals. per hour. By interpolation, lines for any intermediate BMR can be drawn.

diately after eating did not interfere with digestion nor was it improved by a rest pause immediately after eating. Equally conclusive is the work of Hellebrandt, Brogdon and Hoopes (23). Exercise inhibition of the secretion of hydrochloric acid occurs only in the most extreme grades of work.

As suggested above, kidney function presumably depends on work intensity. Benzinger (24) reports that kidney function is reduced from

25 to 75 per cent by work and suggests that diagnostic tests should be carried out only in bed. He gives few details regarding the nature of the work done by his subjects although it appears to have been of high intensity. Van Slyke, Alving and Rose (25) have looked into this question and conclude that their kidney function test is unaffected by moderate activity unless the kidney is less than 50 per cent normal. Schulutko (26) reports that renal water excretion and concentration of various substances in the urine are influenced by hard work, but he obtained no satisfactory measure of kidney function. In a significant study by Chaussin, Laugier and Rousseau (27) it was found that in moderate activity excretion of water, phosphate and other substances may be less while work is going on, but the excretion of urea may be increased slightly. It seems probable on the basis of rather scanty evidence that kidney function is relatively normal in moderate and even hard work in healthy men.

The fuels used in hard work are essentially those supplied in the diet. From one-half to three-fourths of the energy expenditure occurs in four-hour periods after meals. Since digestive processes and activity of the skeletal muscles proceed simultaneously there is little net loss or gain in energy reserves. Disregarding for the moment transformations which may take place, it is evident that the total R.Q. is essentially that of the food eaten. Thus, in the case of the Eskimo's diet of fat, meat and fish the R.Q. in work will be less than 0.8, while the coolie on his diet of rice and fish may have an R.Q. exceeding 0.9. While there may be some slight advantage in reduced energy requirement for digestion and excretion, the principal theoretical advantage of a carbohydrate diet is the economy in oxygen transport. For a given quantity of energy transformed, carbohydrate requires six per cent less oxygen than fat. In addition, Krogh and Lindhard (28) have found that the production of mechanical energy is about one-tenth more efficient from carbohydrate than it is from fat. Accordingly, if the performance of a given task calls for a seven-fold increase in oxygen consumption with carbohydrate for fuel, the increase must be eight-fold with fat. In moderate work the advantage gained by using carbohydrate is of little consequence, but the nearer the worker approaches his capacity, the more important the efficient production of energy becomes.

When it becomes necessary to work in the fasting state the nature of the fuels used depends on their relative availability, the intensity of the work and other undetermined factors. Anderson and Lusk (29) have shown that dogs can carry on moderate work with an R.Q. character-

istic of fat and we have found (30) that a fasting man can maintain a seven-fold increase in MR with fat supplying more than nine-tenths of the energy. The chemical reactions of normal muscular contraction demand the presence but not always the oxidation of carbohydrate unless one postulates the conversion of fat to carbohydrate. There is no doubt that hard work can be accomplished with little more carbohydrate utilization than that available from the glycerol moiety of fats. It is equally certain that in maximal work, as will be seen later, carbohydrate must be available.

The undetermined factors referred to above include the influence of hormones on carbohydrate and fat metabolism—a subject still poorly understood. The early opinion that adrenalin increases carbohydrate utilization was based on studies of blood sugar and R.Q. Later it was found that lactic acid and ketone bodies of the blood increase also. It was suggested that adrenalin might actually reduce carbohydrate utilization and that blood sugar increases because glycolysis is not checked so much as oxidative processes. In our experiments on several subjects in work performed in the fasting state it is clear that adrenalin injection is followed by a greater utilization of carbohydrate together with an increase in lactic acid concentration (30). Over a period in which reserve energy amounting to 5,000 Cals. was utilized the injection of 3 small portions of adrenalin increased carbohydrate utilization more than ½ over that of control experiments. Excretion of acetone bodies was less rather than more after adrenalin.

In recent years it has been suggested that the anterior pituitary produces a hormone concerned with fat metabolism. Anselmino and Hoffmann (31) by injection of aqueous extracts of the dried gland find an increase in acetone bodies of the blood due, it is thought, to increased fat metabolism. Recently Harrow, Chamelin and Mazur (32) have obtained a preparation from urine which appears to disturb carbohydrate and fat metabolism in rabbits. Its injection is followed by hyperglycemia, decreased CO_2-combining power and an increase in acetone bodies and lactic acid in the blood. The uncertain significance of these findings is typical of our ignorance of many phases of the metabolism of fat.

The hormones have other intimate rôles in muscular activity besides regulation of the fuels used. In addition to the specific effects of adrenalin injections on carbohydrate metabolism just referred to, this hormone has been shown by Cannon (33) to be useful for many other reasons in emergencies. The hormone of the adrenal cortex may be concerned

with muscular tone for muscular weakness is one of the most characteristic symptoms of cortico-adrenal insufficiency. Hypertrophy of the rat's adrenal gland with increase in cortical lipoid after exercise has been noted by Andersen (34) and is thought to mean increased cortical activity and perhaps exhaustion of the gland. Eagle, Britton and Kline (35) have reported that the injection of cortin in the normal animal increases endurance. It is doubtful if the well-trained animal has an inadequate supply of this hormone although possibly one of the effects of training is to increase the secretory activity of the adrenal cortex.

Insulin presumably also plays a rôle in exercise. As is well known the blood sugar concentration in moderate as well as in hard work is maintained near the rest level (36). The fact that the balance between the absorption of glucose, its production by glycogenolysis and its oxidation is so nicely adjusted implies that in work as well as in rest the equilibrium between insulin and adrenalin is maintained. Many other interrelations of the endocrines in muscular activity have been discussed by Brouha (37).

That there may be still other unassessed factors which determine the capacity for work is suggested by the report of Lehmann and Szakall (38) that ultra violet radiation effects a 60 per cent increase in capacity for work. If true, this has an important bearing on high altitude studies. Administration of an organic phosphate preparation has a qualitatively similar result according to Atzler and associates (39). There remains some doubt as to whether the effects of training were entirely eliminated. It would be desirable in such experiments to carry on the program long enough after discontinuing medication to insure the attainment of the original state. The lessons of the Hawthorne study (15) lead one to adopt a sceptical attitude regarding the results of short-range studies.

Our more comprehensive knowledge of the metabolism of carbohydrate is perhaps responsible for the impression that it is the universal fuel of muscular activity. Actually there appears little logical basis for such an assumption. While the use of carbohydrate is more economical and is demanded in intense exercise of short duration, in any long-continued fast, with or without work, dependence must be placed on fat. This is true, for example, of hibernating animals and of the camel.[6] As shown by Benedict (41) even man can survive for a month, without extreme acidosis, on his stored energy—chiefly fat. A sugges-

[6] Theoretically the most economical diet for the explorer in barren areas would consist of fat with the necessary minimum of protein, vitamins and minerals. While man cannot tolerate such a mixture, the camel can utilize pure fat. Hedin (40) provided his camels with sesame oil and bran on long journeys.

tion of the rôle of fat in muscular activity is found in the report of Bloor and Snider (42) that the phospholipid content of muscle increases as a result of training.

The possible use of alcohol as fuel in exercise has been discussed by Carpenter (43) and later studied by Carpenter, Lee and Burdett (44) and by Canzanelli, Guild and Rapport (45). Evidently it cannot be used although Troschke (46) thinks that it may replace sugar in non-contractile tissues, thus rendering more carbohydrate available for skeletal muscles.

Aside from its energy-yielding formation in skeletal muscle during anaerobic work, lactic acid is thought to serve other ends in the body. Its direct use as fuel by the heart is clearly shown in studies of the heart-lung preparation by Evans and associates (47). Some years ago it was suggested by Winterstein and Gollwitzer-Meier (48) that lactic acid is produced in the brain in anoxemic intact animals and removed in the lungs. Myerson and associates (49) could not confirm the anaerobic production of lactic acid in the brain in experiments on intact human subjects. Recently Alpern, Simonson, Sirkina and Tutkiewitsch (50) have found additional evidence for the disappearance of lactic acid in the lungs. In 24 post-exercise experiments on 5 dogs arterial blood contained on the average 3 mgm. per cent less lactic acid and about 4 mgm. per cent more sugar than blood from the right heart. This interesting report needs confirmation.

A common characteristic of both moderate and hard work is the early attainment of a steady state. This applies to minute volume, heart rate, blood pressure, respiratory rate and volume, and body temperature.[7] It does not necessarily apply to the rates of oxygen consumption and carbon dioxide production since the mechanical efficiency and respiratory quotient are apt to decrease as the carbohydrate stores are depleted. Nor are any of these functions independent of environmental conditions. The heart rate, particularly, responds sensitively to changes in external temperature and humidity, both in moderate and in hard work. The fact that the Hawthorne study failed to reveal a relation between output and environmental conditions may be attributed to the large factor of safety in such activity.[8] According to our delimita-

[7] A comprehensive study of the inter-relations of minute volume, blood pressure and pulse in dynamic and static work of various intensities has been made by Kagan, Kustanovitsch and Borschewsky (51). These experiments yield useful indices of correlation between hemodynamic and energetic functions.

[8] For our purposes the factor of safety may be defined as the ratio of the maximal increment in oxygen transport to the observed increment in oxygen transport.

tion of moderate work its factor of safety is from 3 to 5. Hence even if there is a decrease of 20 per cent in the oxygen transport capacity because of unfavorable conditions there is still a comfortable reserve in the ability to transform energy.

According to our definition of hard work, its factor of safety varies from 3 to 1. As the level of activity approaches the capacity of the workman, he becomes more sensitive to changes in environmental conditions. Temperature, humidity and air movement are of passing interest to the ordinary employee but are subjects of vital concern to the man doing heavy manual labor. Expressed in another form, a breakdown is imminent when the rate of heat production approaches the heat dissipative capacity of the body.

When an environment unfavorable for heat dissipation leads to breakdown any one of a number of manifestations may be predominant. One which has been of particular interest is commonly referred to as heat cramps.[9] This condition may develop after an excessive amount of sweating while doing hard work. It has been appreciated since the work of Moss (53) that the loss of salt in the sweat is an important factor. The studies of the English school led them to suggest that the essential features of heat cramps were loss of sodium and potassium chloride solutions in sweat, the replacement with water and an associated decrease in osmotic pressure. The remedy proposed was an adequate provision of salt in the food and the addition of sodium and potassium chlorides to drinking water. While the remedy was entirely effective the analysis of the course of events is now known to be inaccurate.

Studies carried out in dry heat at Boulder Dam on members of our party (54) revealed that excessive sweating depletes the reserve of sodium chloride but never produces any deficiency of potassium. Furthermore, as the dilute salt solution is lost in the sweat one drinks voluntarily only enough water to maintain a normal concentration of salt in the blood. It was found possible to continue for several days with excessive sweating, with virtually no sodium chloride in the urine, with relatively normal chloride concentration in the blood but with a deficiency in body weight as great as 2 kgm. A voluntary increase in water intake is followed promptly by elimination of the excess by the kidneys but with little flushing out of sodium chloride. However, the normal weight can be restored by drinking a suitable amount of 0.9 per cent NaCl solution. Experiments of a related character and with similar results have been carried out by Cassinis and Adilardi (55).

[9] This subject has been discussed and the literature reviewed comprehensively by Talbott during the past year (52).

Workmen who experience cramps have undergone an even greater depletion of salt than that experimentally produced in ourselves (56). Not only is the urine free of chloride, but the concentration of sodium and chloride in the blood is lowered, sometimes as much as 10 per cent. The body weight may be reduced as much as 4 kgm. because of inability to retain water without salt. Striking evidence of dehydration is found in the high concentration of serum protein. Dehydration is not confined to the blood but exists to a corresponding extent in the tissues. Proof that "water intoxication" does not exist is two-fold. First, direct determination of serum osmotic pressure reveals no unusual departure from normal even in severe cases.[10] Frequently values after recovery may be lower than when cramps are maximal. Second, prompt and complete relief of symptoms follows the injection of 0.9 per cent salt solution, a procedure which does not increase the osmotic pressure of body fluids.

The mechanism of heat cramps requires further study for its complete elucidation. Clearly it is not a question of osmotic pressure, as we have shown. Nor does it seem to be a question of sodium-potassium ratio for the injection of isotonic sodium bicarbonate is not a remedy.[11] The greatest uniformity is the simultaneous increase in serum protein and decreases in sodium and chloride concentrations in the blood. Medication tending to restore all three of these to normal brings relief and no other medication tried is effective. It is hoped that further study will lead to a clearer understanding of the situation.

Another manifestation of inability to carry on in a hot environment is heat exhaustion or heat stroke. There appears to be no precise differentiation between these conditions although heat stroke is the more acute condition. Some cases of heat stroke have such extraordinarily high temperatures as 110 to 112°F.; these do not usually survive. Pathological conditions in the circulatory system are predisposing. Inactive persons—particularly the weak and infirm—are the characteristic victims although heat exhaustion is about as frequent in the heavy industries as heat cramps.

The experimental production of heat exhaustion in man would be, for the subject, an unpleasant and possibly a dangerous procedure. We have found it possible, however, to produce a situation which must bear

[10] Unpublished observations.

[11] It is interesting to note in this connection the report by Arden (57) that the ingestion of 15 grams of potassium chloride produces many unpleasant results including prostration, nausea, abdominal pain, loss of power in the limbs, paresthesia but no cramps, diarrhea or vomiting.

close similarity to the early stages of heat exhaustion (58). With a room temperature of 35°C., a humidity of 50 per cent and virtually no air movement conditions similar to hot sultry days of summer were simulated. With a work rate about 8 times the resting MR, i.e., at the upper limit of hard manual labor, we found a breakdown usually occurs within an hour. In every instance the heart rate was much higher than when the same task was carried out in a room at 12°C. The body temperature may or may not be much higher, the blood lactic acid is only moderately increased, and the volume and composition of expired air are little different. The constancy of most physiological functions under these circumstances is proved by the work of Liberson and Marquès (59). The critical factor is evidently circulatory. The increased demand for heat dissipation requires the movement of a considerable fraction of blood through peripheral channels. Its rate is increased by the opening of an unusual proportion of capillaries as well as of the arterio-venous anastomoses as described by Grant and Bland (60). The capacity for transporting oxygen to the tissues is reduced and the factor of safety becomes dangerously small. Breakdown may occur because the circulatory system is no longer able to satisfy the conflicting demands for oxygen supply and for heat dissipation.[12]

The discomfort arising from difficulty in heat dissipation may be demonstrated strikingly by the use of clothing relatively impervious to air and water vapor. Figure 2 shows the effects on the heart rate of moderate exercise with various handicaps placed on the heat-dissipative mechanism. The subject wore heavy woolen clothing, carried a 40-lb. pack, and walked for three consecutive 50-minute periods with intervening 10-minute rest periods. Perhaps the most striking feature of this series of experiments is the cumulative effect on the heart rate. Despite the constancy of oxygen consumption, the relative constancy of body temperature, and uniformity of experimental conditions, the heart rate increases in all experiments. The states of most variables concerned were as notable for their constancy as is that of the pulse for its lack of constancy. Trends in heart rate which might have been considered of minor significance had the experiments been terminated in 20 minutes became a major phenomenon by the end of three hours.

Maximal work. The states which have just been discussed fall within

[12] The intimate relation between external temperature and heart rate of men at work is demonstrated by the work of Herxheimer (61). The fact that patients with heart disease cannot carry unusual burdens on the circulatory system accounts for their sensitivity to heat.

the transition zone between hard work and what may be described as maximal work. It is useful to consider this third type of activity as including all work which is more intense than can be continued at a uniform rate for the regular working day, no matter how favorable the environmental conditions. Few jobs in modern industry are in this category, although the work of those steel men who have equal alternating periods of rest and activity are typical of it. The forced march

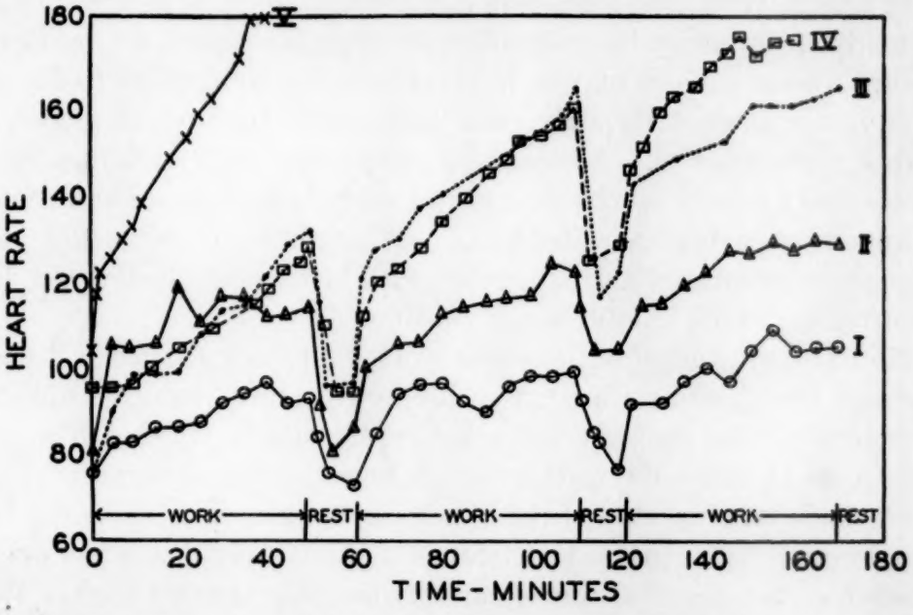

Fig. 2. The effect of restricted evaporation of sweat on the heart rate. The MR in experiments I to IV is about 3 times basal and in V, 6 times basal. The conditions were as follows:

I. Heavy woolen clothing worn.
II. Face mask and rubber gloves in addition to woolen clothing.
III. Oilskin suit worn over woolen clothing.
IV. Face mask, rubber gloves and oilskin worn in addition to woolen clothing.
V. Similar to IV except for higher grade of work.

or the charge in the field of war exemplify maximal work. Most of our examples, however, are found in the field of sport.

A normal man carries on moderate and even hard work with minor changes in the properties of his blood. Lactic acid concentration and alkaline reserve are unchanged. The volume of air breathed is delicately adjusted to maintain the normal concentrations of free oxygen and carbon dioxide in arterial blood (62) (63). Consequently, the percentage saturation with oxygen and the bicarbonate content differ little, if at all, from the resting values. Venous blood is more acid than in

rest because the rate of blood flow increases more slowly than the metabolic rate; as a consequence, venous blood carries a greater load of carbon dioxide and less oxygen than in rest. Throughout the entire range of moderate and hard work the heart rate, respiratory volume, and circulation rate are nearly linear, though different, functions of the metabolic rate. Studies in this field have been made by Bock and associates (64) and by Schneider (65).

In passing from hard work to maximal work, we enter an area in which a steady state cannot be maintained or never is reached; i.e., work ends in breakdown. There may be hyperpyrexia due to inability to dissipate heat fast enough. Hypoglycemia may result because of inadequate carbohydrate reserve. A rising heart rate may mean circulatory failure. In the most intense work the oxygen supply is inadequate and reserves of anaerobic energy are called upon. When these are exhausted, major changes in arterial blood, associated with increases in lactic acid, have occurred and work can no longer continue.

Noteworthy studies on maximal work have been carried out during the past few years by Christensen (66). His sturdy subjects were able to maintain what we may call a temporary steady state with work as intense as 15 times the resting metabolic rate while observations were being made on properties of the blood, respiration, circulation and body temperature. One interesting feature of these experiments is a demonstration of the essential rôle of carbohydrate in maximal work. When his subjects were on a high carbohydrate diet, work of a grade which could be continued as long as 4 hours ended with a breakdown because of stiff and sore joints. On a high fat diet and other conditions unchanged exhaustion forced a halt after 90 minutes of work. Hypoglycemia and subjective sensations indicated that the demand for carbohydrate had become acute. The administration of glucose brought relief promptly; within a few minutes the subject was able to resume work. The handicap of a low carbohydrate diet has also been shown by Gemmill (67). One hour of maximal work was followed by increase in acetone bodies in the blood.

This evidence adduced by Christensen, and our own experiences (68) indicate that, while moderate work can be carried on for long periods on a fuel chiefly fat, there is a critical intensity of work above which carbohydrate becomes important: the higher the level of activity the more carbohydrate is required. As has been pointed out above, the work of Anderson and Lusk (29) proves that dogs can do moderate work on fat alone, and our own experiments show that dogs can work at high

levels on nearly pure fat. In extreme cases, however, the dog, like man, requires a supply of carbohydrate (68). Whether this emergency demand for carbohydrate arises from a general need by skeletal muscle or from some specific need, as, for example, by the heart, brain, or liver, is uncertain.

That selective utilization of carbohydrate is characteristic of maximal muscular work is now generally accepted. The report of the Anglo-American Pike's Peak expedition (69), the investigations of Hill and associates (70), the Benedict and Cathcart monograph (71), and many studies from this laboratory (64) (72) (73) all point in this direction. Krogh and Lindhard (28) have been reluctant to accept this conclusion because their studies on moderate work led them to believe that "*both during rest and during work the proportion of fat to carbohydrate catabolized is a function of the available supplies of these substances.*" While this conclusion as stated is true it is now certain that the function is not necessarily the same in rest and in work. The experiments of Christensen have convinced Krogh and Lindhard, for in a recent report these three authors (74) state that in very heavy work "*a definite and real rise in quotient will take place as compared with the previous period of rest.*" Included in the experiments reported by Christensen was one in which a subject reached a steady state with an oxygen consumption of 4 liters per minute and with an R.Q. of 0.95.

Under other circumstances Christensen found that incompetence in dissipating heat may be a limiting factor. The body temperature in extremely hard work of 30 minutes' duration may reach 40°C.; beyond this continuation of work is dangerous. This type of breakdown and that involving carbohydrate depletion are characteristic of the strong man; no ordinary person will push himself far enough to produce hypoglycemia or hyperpyrexia except in great emergency.

A third type of breakdown has been studied in our laboratory recently. Without a serious rise in body temperature and with normal blood sugar, work which has been carried on in a comfortable environment easily for two hours may rather abruptly become difficult. Simultaneously the heart rate departs from its plateau value and approaches the maximum in a climactic manner. The subjective sensations are quite uniform. For the first two-thirds of the period the work seems easy,—"*I can keep this up all day*" is a typical remark. One-half hour before the finish the subject finds the work is harder but thinks he can last another hour. During the last 10 minutes he watches the clock, astonished at his sudden loss of ability to carry on.

A rapidly rising heart rate would seem to imply circulatory failure. Yet it is interesting to note that in such circumstances there may be little increase in lactic acid. It will be recalled that a similar type of breakdown occurs when exercise ordinarily easy is attempted in a hot humid environment. There also the heart rate rises to the maximum, frequently with only a moderate elevation in body temperature and with only a moderate increase in lactic acid. According to current theories, a deficiency in oxygen results in lactic acid accumulation. Either this idea is unsound or our supposition that circulatory breakdown is imminent in these two circumstances is incorrect. This question requires further consideration.

The marathon presents an excellent example of maximal work of the sort in which lactic acid increase is unimportant but in which the limiting factors revealed by laboratory experimentation are illustrated. Hyperpyrexia in runners after finishing has been reported by Savage (75). Extreme hypoglycemia has been found by Gordon and associates (76) and Best and Partridge (77). Breakdown of essential units in the machine, such as that reported by Christensen in long-continued experiments, is a familiar feature of the race. Defeat is often charged, for example, to blistered feet. Although direct evidence is lacking, it is probable that the heart must work near the limit of its capacity. Finally, in contrast with work experiments without emotional stimuli, the nervous and hormonal influences may play a great rôle. Such stimuli may be partially advantageous, partially disadvantageous. For example, some men are unable to eat or sleep normally before a race. Viewed as a mechanical problem, the ingestion of a heavy carbohydrate meal a few hours before the race and a supply of glucose before and during the race are logical desiderata. But the stomach of a nervous runner may not be able to tolerate such a rational procedure.[13]

These considerations bring to attention the large individual differences revealed by the performance in maximal activity. Success in a marathon, for example, depends on *a*, capacity of the heart and skeletal muscles for sustained work; *b*, efficiency with which work is carried out; *c*, reserves of fuel: fat should be minimal because of the handicap of carrying excess weight, while carbohydrate should be maximal because of the economy gained in the oxygen requirement; *d*, capacity for dis-

[13] Wishart (78) has had the opportunity to study the response of a long distance cyclist who is a vegetarian. The best performance was on a diet containing much milk and eggs. He concludes that bulk and palatibility are important considerations in men doing heavy work.

sipating heat; *e*, integrity of essential units, and *f*, coördination by the nervous and hormonal agencies. When one considers that many runners observe training rules based on superstitition and prejudice rather than on these facts, their performances seem all the more remarkable.

We come finally to maximal activity in which a significant fraction of the work is carried on anaerobically. We owe to Hill most of our knowledge in this area (70). Into that region of an unattainable steady state where conservative physiologists had feared to go, Hill and his companions advanced boldly. His conception of the contraction and payment of the oxygen debt, as well as the beautiful experiments in which the debts were evaluated, have provided a sound basis for further investigation.

As in every other advance in physiology, some error was mixed with the truth. The results were interpreted in terms of the then current lactic acid theory of muscular contraction. Hill saw that two processes were involved in the payment of the oxygen debt, a rapid one complete in a few minutes and a slow one requiring in extreme cases 80 minutes or more. It was suggested that the rapid process involved either oxidation or resynthesis of lactic acid which had not escaped from the muscles, while in the second stage the remainder is disposed of. According to the theory any increment in metabolic rate is accompanied by an increment in lactic acid.

Evidence soon accumulated that in moderate work blood lactic acid remains at the rest level. Thus it was shown by Bock and associates (79) that the alkaline reserve (in exercise, a reciprocal function of lactic acid) does not vary with metabolic rate until some critical level of metabolism is reached. Since then blood lactate determinations in this laboratory (72) (73) (80) and by Owles (81) have been fully confirmatory.

It has been an attractive theory that the level of lactic acid is a function of the concentration of free oxygen in the tissues or in the extracellular fluid. Early reports by high altitude investigators seemed to support this idea (82). It was a surprise to find, however, that when a low arterial saturation is produced in normal man by breathing a low oxygen mixture, the resting value for lactic acid remains unchanged (83). It was even more surprising to learn from the work of Weiss and Ellis (84) that patients with considerable decompensation in heart disease have virtually normal lactic acid concentration in the blood in rest and only slightly greater elevation of lactic acid than normal subjects doing the same standardized work. Patients barely able to perform the task and

who finished with intense dyspnea had the same lactic acid increase as normals who did the task with ease.

Our Leadville party (85) at a barometric pressure of 525 mm. found that for a given metabolic rate the level of lactic acid reached was higher than at sea level. By extrapolation from this finding we had supposed that, while the maximal metabolic rate decreases with altitude, a constant limiting value for lactic acid in anaerobic work would be reached at all elevations. During the past summer Edwards (86) had an opportunity to test this theory at higher altitudes and found it wrong: at high altitudes in work on the ergometer or in climbing it is impossible to reach as high a lactic acid concentration in work as at sea level. Immediately after two men had finished a climb from 18,400 up to 20,000 feet with maximum exertion during the last hundred feet their blood lactic acid concentrations were 1.7 and 1.8 mM. per liter. On several subjects at rest at this elevation the values were all within the sea level range despite arterial saturations of from 55 to 70 per cent.

A comparison of our experiences at high altitudes with those of patients with heart disease (84) reveals many points of similarity. Breathlessness comes from slight exertion and the maximal metabolic rate is much below the normal maximal value. The volume of air breathed is much greater for a given metabolic rate than in a normal state. In exhausting exercise there is a much smaller change in alkaline reserve, in lactic acid, and in arterial pH than in the normal man after severe exercise.

The facts so far presented are based on analysis of blood and do not disprove Hill's theory that lactic acid under some circumstances may be held back in the muscles. Even when it was shown by Davenport and Davenport (87) that the resting value for lactic acid concentration in tissues is about the same as in blood,[14] there was still the possibility that affairs might be different in exercise. The question seemed to be settled, however, by Margaria and Edwards (89). They were able to show that the rate of removal of lactic acid from the entire bodies of mice after stimulation was the same as from the blood of man. In other words, there was no evidence for an initial rapid rate of removal during the first few minutes of recovery.

[14] In great contrast are the observations of Jokl (88). He finds that muscle has from 4 to 6 times higher concentration of lactic acid than blood whether in rest or after work. Samples were obtained by killing the animal without an anesthetic; whether or not such a gradient of lactic acid is characteristic of the living animal must be determined by further study.

Experiments by Dennig, Talbott, Edwards and Dill (91) carried out two years before had led them to suggest that more anaerobic work might be done if the capacity for neutralizing lactic acid is increased by establishing an initial alkaline state. This suggestion was based on the observation that in exhaustion after anaerobic work about the same lower limit of alkaline reserve is reached and on the assumption that oxygen debt is a function of lactic acid. A test of this hypothesis by Dill, Edwards and Talbott (90) showed that with an initial alkalosis the concentration of lactic acid in the blood after working to exhaustion might be 40 per cent higher than normal, but the oxygen debt was no more than 20 per cent greater. Later a practical test was given the hypothesis. For this we have to thank Coach Farrell and members of the Harvard track squad. On the day of the spring time trials each runner took 20 grams of sodium bicarbonate at lunch time. No observations were made on the blood, but judging by laboratory experiences the alkaline reserve must have been significantly elevated at the time of running. Yet none of these men made outstanding records as compared with previous performances, and some thought they were handicapped. The possibility of producing a useful increase in capacity for accumulating an oxygen debt seems rather remote.

The mechanisms involved in contracting and paying the oxygen debt have been investigated more fully by Margaria, Edwards and Dill (92). They found that in a trained runner no lactic acid appears in the blood up to a rate of work corresponding to about two-thirds of the maximal metabolic rate, after which the lactic acid increases very rapidly, with an estimated increment of 7 grams for each liter of oxygen debt. The removal of lactic acid is a very slow process which can be represented by an equation of the form.

$$L = L_r + 10^{a-bt}$$

where L is the lactic acid concentration at time t, L_r the concentration of lactic acid in the body at rest, 10^a the extrapolated value of excess lactic acid concentration in the blood at the beginning of recovery and b the velocity constant. In different individuals and under various conditions the velocity constant differs little from the value 0.02; in fact, the constant had the same value for the disappearance of lactic acid from the bodies of mice, as shown by Margaria and Edwards (89).

It was further concluded by Margaria, Edwards and Dill (92) that the oxygen consumption curve during recovery depends on four proc-

esses: *a*, the basal oxygen consumption; *b*, a slow process of lactic acid removal; *c*, a rapid process nearly complete in 5 minutes, and *d*, a process which may continue for several hours and which for the present cannot be exactly defined. The third process is characteristic of moderate and all grades of hard work in which no lactic acid is produced; it is termed *alactacid* by Margaria and is presumed to involve the resynthesis of phosphocreatine. According to his calculations the maximum alactacid debt in the subject studied was 2.5 liters of oxygen, while the lactacid debt was twice as great. It was noted that the disappearance of lactic acid from the blood at the beginning of recovery after strenuous exercise shows a lag of 6 to 8 minutes' duration. This does not seem to be fully explained by slow diffusion of lactic acid from muscles to the blood, by an initial slowness of oxidation or by a delayed lactic acid production. Yet it seems likely that the last alternative may be of some importance, that is, the alactacid debt at the end of exercise may considerably exceed 2.5 liters and in the first process of rapid payment part of it is converted to lactacid debt. This possibility fits in with the observation that the greater increment in lactic acid made possible by establishing an alkaline state is accompanied by a relatively small increase in oxygen debt.

The properties of the blood in anaerobic work cannot be described adequately in the brief space remaining. Arterial blood becomes more and more acid in reaction, the decrease in CO_2 tension not keeping pace with decrease in bicarbonate consequent upon the appearance of lactic acid. The rising osmotic pressure in tissues draws water from the blood, thus concentrating the serum proteins and red cells (93), (94). Although the oxygen tension may change but slightly, the percentage saturation is lowered because of displacement to the right of the oxygen dissociation curve (79). Many associated changes are discussed elsewhere (8), (95). Venous blood may be nearly arterial in character if drawn from a superficial vein from the arm if work has been done by the legs, particularly if the external temperature is high. The anastomoses in the hand which have been described by Grant and Bland (60) permit a rapid flow with little oxygen utilization. On the other hand, if blood is drawn from the femoral vein after a subject has run to exhaustion, it may be found nearly free of oxygen, with a CO_2 tension as high as 100 mm. and with a pH as acid as 6.9.[15] To make the story still more

[15] Laug (96) reports that the pH of venous blood from the arm may be as acid as 6.95 some minutes after a quarter-mile run. If blood had been obtained from

complicated, both venous and arterial blood are apt to change rapidly in composition during the transition from work to rest. It is necessary to use caution, therefore, in the assumption that blood samples drawn in recovery give a safe index to its composition in work.

While there is little important alteration in the composition of arterial blood of man in moderate work, the changes may be very great in the dog. This is due in part to the fact that the dog depends on breathing not only for gas transport but for heat dissipation (97). Under unfavorable conditions the volume of air breathed may be increased out of proportion to the rate of CO_2 production. Tensions as low as 10 mm. have been observed by Rice and Steinhaus (98) and it has been shown by Schlutz, Hastings and Morse (99), that the physicochemical changes in the blood depend on the nature of the activity. Hastings and Steinhaus (100) have developed a useful graphical method of representing the relation between three properties of the blood which may undergo great change in exercise: pCO_2, combined CO_2 and pH.

A familiar aspect of severe anaerobic work is the need for "warming-up." This is generally recognized by trainers and athletes but the proof that it is a real phenomenon has been given only recently. Christensen, Krogh and Lindhard (74) report that by step-wise increase in intensity of work one of their subjects can reach an oxygen intake of 5 liters per minute, but without warming up his maximum is 4.6 liters. This fact fits in with observations that the pulse-rate of man is slow in reaching its maximum value. In contrast with the slow acceleration of the pulse in man, the pulse of the dog may become maximal in 5 or 10 seconds (68).

In dog-racing no opportunity is given for warming up and there may be no need of it. The records for greyhound racing indicate that the

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the femoral vein, he might have found even greater acidity as the following unpublished experiment shows:

Duration of work: 65 seconds.

Composition of femoral venous blood in recovery

TIME AFTER END OF WORK	TOTAL CO_2	pCO_2	pH_a	LACTIC ACID
seconds	*mM per l.*	*mm. Hg*		*mM per l.*
48	21.7	100	6.92	12.7
130	16.5	78	6.91	12.7
350	12.5	53	6.98	13.7

maximal rate is reached over the quarter-mile distance but there is little slowing until the distance exceeds one-half mile. The published American records correspond to the following rates, expressed in miles per hour:

DISTANCE	MAN	GREYHOUND	RATIO
yards			
330	20.2	37.1	1.84
440	19.0	37.2	1.96
550	17.8	36.1	2.03
880	16.4	34.4	2.10
990	15.6	30.2	1.94

In addition to species difference there may well be age difference in the lability of the organism. While it is likely that youth is more competent than age in making sudden adaptations, laboratory evidence on this point is scarce. Of great interest in this connection is the study by Fessard, Fessard and Laugier (101) of the heart rate in relation to age. His subjects, varying in age from 6 to 17 years, ran 50 meters and the heart rate was measured before and immediately after work. There was the well-known diminution of resting rate with age, but surprisingly enough the greatest increase in rate as a result of exercise was in the older subjects. In other words, the rates observed after work were more nearly equal than the resting values.

An interesting controversy has developed regarding the efficiency of effort of short duration. Simonson and Hebestreit (102) report that work lasting one minute may be only one-half as efficient as work of 7 minutes' duration. This conclusion has been challenged by Hansen (103) and Crowden (104), who found little relation between efficiency of work on the bicycle ergometer and its duration. Simonson and Sirkana (105) reply that such exercise seems to be exceptional in this respect. It is chiefly dynamic and has a high efficiency. In weight-lifting and other types of more or less static activity they substantiate their earlier results. The method Simonson used involves measuring the excess oxygen in work and recovery. Part of this increase may be a consequence of glandular stimulation and not of anaerobic processes of work. As Margaria, Edwards and Dill (92) have shown, one-third or more of the excess oxygen used in recovery may be due to the increase in resting metabolism. The importance of this phenomenon in Simonson's experiments is unknown but it is possible that the low efficiencies in work of very short duration are explained by stimulation of the resting

metabolism out of proportion to the duration of activity. A similar idea is expressed by Herxheimer (106) as follows:

Kurz, ich halte es für möglich dasz das "Debt" wenigstens für die reine steady—state Arbeit, keine Schuld ist, sondern eine—für die Arbeits—energetik bedeutungslos—Luxuskonsumption darstellt, die zum Arbeits schlusz gehort wie das Auslaufen eine abgestellten Maschine.

Space does not permit more than brief reference to other subjects although one, intimately concerned with all types of activity, deserves special attention. The happiness, the performance and the nutritional state of the worker are indissolubly linked. It is important, therefore, that his diet be adequate not only in calories but also in vitamins. Asthenia is characteristic of animals suffering from vitamin deficiency even with forced feeding. Of all the vitamins, the B complex seems to be most directly related to muscular activity. Impairment of the ability to remove lactic acid from the blood and tissues, especially after exercise, is characteristic of vitamin B_1 deficiency in pigeons and men. Notable investigations in this field are those of Collazo (107), Collazo and Morelli (108), Inawashiro and Hayasaka (109), Hayasaka (110) and Fisher (111). There is an accumulation of lactic acid, especially in the brain stem, in pigeons showing the terminal symptoms of B_1 deficiency according to Kinnersley and Peters (112). These facts associate beriberi with lactic acid metabolism. Of broader interest is the fact that poorer sections of many communities live in danger of deficiency in one or another of the vitamins. Reference may be made to Wilson (113) for studies of Egypt; McCarrison (114) for India; Goldberger (115) for the United States; and to reports of the Medical Research Council (116) for England.

Little attention has been paid in this review to the chemical processes going on within the muscle except in so far as these are revealed by properties of the blood and responses of the intact organism. The more purely chemical questions have been reviewed by Eggleton (117). Interesting developments in the field of muscle chemistry include studies of myoglobin by Theorell (118); the rôle of glutathione by Wachholder and Quensel (119); the viscous-elastic properties of muscle by Furusawa, Hill and Parkinson (120), Fenn (121) and Cattell and Edwards (122); and photoelectric measurement of pH changes in the contracting muscle by von Muralt and Margaria (123). Some light is cast on the physical structure of the muscle fibre by the studies of Edsall (124) and of Edsall and von Muralt (125) (126).

SUMMARY

In this account of the economy of muscular activity arbitrary limits have been set to moderate work, hard work and maximal work. In the first category the amount of energy transformed is small, the effects of the physical environment almost negligible, and if fatigue occurs it is of remote rather than of direct interest to the physiologist. With work approaching the limit of capacity, environmental conditions become more important. Fatigue appears to depend on the ratio of the work done to the capacity for work. In maximal work a temporary steady state may be attained, but a breakdown eventually occurs for which any one of several functions may be responsible. The most intense work is chiefly anaerobic and here no steady state is reached. Fatigue increases with the rapid depletion of energy reserves and the accumulation of unoxidized end-products.

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THE PHYSIOLOGICAL ACTIVITY OF NUCLEIC ACID AND ITS DERIVATIVES

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Although a considerable amount of work has been undertaken upon the biochemistry of nucleic acid and its derivatives, the physiological activity of these substances has only recently been appreciated. The knowledge already gained is considerable, but it is by no means possible to make a coherent story of the results obtained.

In studying the physiological activity of nucleic acid and its derivatives a considerable number of substances must be tested; for nucleic acid is a complex body and gives rise to many breakdown products. It is not proposed to consider the biochemical aspects of these various breakdown products or whether each or every substance is known to exist or is likely to exist in the animal body. This aspect has been most ably dealt with by Jones (1920), Feulgen (1923) and Levene and Bass (1931), and additional data are daily accumulating. It is important nevertheless for the reader to have some idea how the various substances are related one to another. For this purpose it is perhaps sufficient to enumerate the substances which can be obtained from nucleic acid either by enzymatic or chemical breakdown and to indicate the manner in which these substances are interrelated.

Nucleic acids are of two types, plant and animal, of which yeast nucleic acid and thymo-nucleic acid are examples. There is no doubt that the two nucleic acids have a very similar constitution, although there is a slight difference in the nature of their ultimate hydrolytic products, for intermediate decomposition products can be obtained from the plant acid similar to those from the animal nucleic acid. All the ultimate hydrolytic products of both nucleic acids have been identified. Both nucleic acids yield two purine bases, two pyrimidine bases, phosphoric acid and sugar. The purine bases adenine and guanine, the pyrimidine base cytosine and phosphoric acid are obtained from both nucleic acids. The additional pyrimidine base that is found in the plant acid is uracil, in the animal acid the corresponding one is thymine.

These two bases, though not the same, are closely related. The sugar from the plant acid is d-ribose and from the animal acid a d-ribodesose. All the bases when united to a sugar form substances called mononucleosides, and if phosphoric acid is linked to these mononucleosides, mononucleotides are obtained.

Four such mononucleotides are apparently linked together to form the complex molecule nucleic acid. In yeast nucleic acid they are:

1 phosphoric acid—sugar—adenine (adenylic acid)
2 phosphoric acid—sugar—guanine (guanylic acid)
3 phosphoric acid—sugar—cytosine (cytidylic acid)
4 phosphoric acid—sugar-uracil (uridylic acid)

In animal nucleic acid the uracil is replaced by thymine. It is possible to break off the phosphoric acid from the mononucleotides, and obtain four mononucleosides. Each contains a sugar and a base only

1 sugar—adenine (adenosine)
2 sugar—guanine (guanosine)
3 sugar—cytosine (cytidine)
4 sugar—uracil (uridine)

and again in animal nucleic acid the uracil is replaced by thymine. From these four mononucleosides the sugar can be split off and the bases themselves obtained, namely, the pyrimidine bases cytosine and uracil (or thymine) and the purine bases adenine and guanine. If these two latter bases are deaminated hypoxanthine and xanthine are formed. Deamination, however, can be brought about before either the phosphoric acid or the sugar is split off so that two new compounds are obtained

1 phosphoric acid—sugar—hypoxanthine (inosinic acid)
2 phosphoric acid—sugar—xanthine (xanthylic acid)

The removal of the phosphoric acid from these substances leaves two nucleosides,

1 sugar—hypoxanthine (inosine)
2 sugar—xanthine (xanthosine)

and from these the sugars can be split off to leave hypoxanthine and xanthine both of which can be oxidised to form uric acid. In this manner a number of closely related substances of varying complexity can be obtained, and they form the nucleic acid, group of substances which have been tested in respect of their physiological activity. An additional substance, muscle adenylic acid has also been largely investigated. It was discovered by Embden and Zimmermann (1927) during their studies on the phosphoric esters of muscle and isolated

from muscle tissue. It is not yet decided whether this mononucleotide is identical with the similar mononucleotide derived from other organs or from animal nucleic acid. As far as muscle and yeast adenylic acids are concerned they both yield the same adenosine, but the two acids have very different rates of hydrolysis. There can be no doubt that the two acids are isomers, and the difference can only lie in the allocation of the phosphoric acid on the nucleoside. Although muscle adenylic acid has been isolated from muscle in considerable amount it exists as such in the normal tissue in extremely small quantity (Cori and Cori, 1931). From skeletal muscle a higher phosphorylized compound (Lohmann, 1931) adenyl triphosphate has been isolated. In the heart a more complex body which seems to be a compound of adenosine di- and adenosine triphosphoric acid (Ostern, 1934) has been identified. The muscle adenylic acid which has been isolated both from skeletal and heart muscle is apparently formed by the cleavage of the higher compounds during the preparation of the muscle mash. The phosphate radicle of the higher phosphorylized compounds is therefore in part readily removed but that of muscle adenylic acid is relatively stable. It is possible to deaminate chemically adenyl triphosphate and so to obtain inosine triphosphate (Gillespie, 1934). This substance has also been the subject of a few investigations. In addition to the pyrimidine derivatives obtained normally from nucleic acid vicine has also been tested on a few occasions. It is a hexose nucleoside. The basic compound is a pyrimidine derivative divicine united to a sugar dl-glucose. Another pyrimidine derivative that also has been tested is 4, 5-diamino-2, 6-dioxypyrimidine. This substance is closely related to divicine but NH_2 groups are disposed differently around the pyrimidine ring. It contains no sugar molecule.

THE PHYSIOLOGICAL ACTIVITY. Before proceeding to detail the physiological observations which have been made, it would perhaps be helpful at this stage to summarise the chief actions which have been noted. The active products slow the heart, and lower the blood pressure. They inhibit the intestinal muscle, and stimulate the uterus. They produce a leucocytosis.

ACTION UPON THE SPECIALISED TISSUE OF THE HEART. It is important to keep in mind that three main effects are observed when the heart is under the influence of the nucleic acid derivatives. These substances not only influence the specialised tissue such as the sino-auricular node (pacemaker) and the auriculo-ventricular bundle but also the muscle itself and the coronary vessels. It is proposed, however, at this stage to consider only the action upon specialised tissue.

It was the effect upon the auriculo-ventricular bundle of the guinea pig's heart that first called the attention of Drury and Szent Györgyi (1929) to the physiological activity of these derivatives. They were interested in a substance present in heart muscle extracts which produced a temporary heart block when introduced intravenously into the guinea pig. Using this biological test to determine the presence of the substance they finally isolated it in crystalline form and found it to be muscle adenylic acid. The difficulties which attended this preparation and the small yield of substance obtained rapidly drove them to search for closely allied substances more readily procurable. Adenosine obtained from yeast nucleic acid was first tried and found to have a similar action. Although the main effect is exerted by these substances upon the auriculo-ventricular bundle, the sino-auricular node is also affected, for the sinus rhythm is definitely slowed during the period of the heart block. In the guinea pig therefore there is depression of sinus activity and of conduction through the bundle; the latter being more intense than the former. De Caro (1931) reported bradycardia in the guinea pig using muscle adenylic acid. A considerable number of related substances were tested by Drury and Szent Györgyi at this time, namely: yeast nucleic acid, thymo-nucleic acid, guanylic acid, inosinic acid, guanosine, adenine and guanine. As they failed to produce heart block they were stated to be inactive, but later work showed that this was not strictly true, the doses used having been insufficient. If amounts ten to twenty times greater than that at which adenosine was effective were used yeast adenylic acid and adenine produced heart block but guanosine, guanine and inosinic acid still proved to be inactive (Bennet and Drury, 1931). Recently Drury (1934) has noted that adenyl triphosphate produces heart block in the guinea pig and appears to be more active than adenosine. When other mammalian hearts were tested, the most usual effect was a depression of the sino-auricular node leading to a slowed beat without heart block. For instance, Drury and Szent Györgyi (1929) found that in the intact anesthetised cat, rabbit and dog, muscle adenylic acid and adenosine slowed the heart considerably but the beat originated in the sinus and spread normally through the heart. In the dog they were able to show that there was a depression of conduction through the auriculo-ventricular bundle, but that this was counter-balanced by the slowing of the heart. In these animals the more intense action is upon the sinus though there is a slight action upon the *A-V* bundle. In the intact rat the sinus activity is inhibited and the heart beats slowly in response to a rhythm initiated in the *A-V* node (Drury, 1932b). Wedd (1931) using the

perfused rabbit's heart found that the heart was slowed by adenosine and muscle adenylic acid, but that yeast adenylic acid was almost ineffective. Bennet and Drury (1931) obtained heart block in the perfused guinea pig's heart with adenosine. Wedd and Fenn (1933) noted that yeast adenylic acid has little or no influence on the excised auricles of the guinea pig or rabbit, but that adenosine definitely slowed the rhythm. Gillespie (1934) speaks of "heart block" in the rabbit's heart perfused with adenosine triphosphate, inosine triphosphate, muscle adenylic acid, inosinic acid and adenosine. It is difficult to decide whether he is dealing with a simple slowing of the whole heart or a "heart block" but he is evidently dealing with a depression of the specialised tissue, and finds inosinic acid to be active, which Drury and Szent Györgyi (1929) found was inactive. Cytidylic acid (Drury, 1932b) has no influence upon the guinea pig heart, but produces a bradycardia in the rat.

Upon the human heart the action is apparently variable. Honey, Ritchie and Thomson (1930) injected adenosine into subjects with a normal cardiac rhythm and noted impaired conduction between auricle and ventricle with dropped beats. They also speak of a slowing of the whole heart before the dropped beats occurred. Rothmann (1930) using muscle adenylic acid obtained a definite depression of the sinus rhythm, and no evidence of auriculo-ventricular block. Hartmann (1932) using the same derivative found that the heart rate was increased initially and subsequently was decreased slightly. On a few occasions the conduction between auricle and ventricle was depressed. Richards (1934) using adenosine, yeast and muscle adenylic acid found that the heart rate invariably quickened and that in about 25 per cent of the subjects tested the auriculo-ventricular conduction was disturbed. The two adenylic acids were equally active on the pacemaker and bundle tissue.

The action upon the cold blooded appears to be similar to that upon the mammalian heart, a simple slowing without heart block being the usual result. Parnas and Ostern (1931, 1932) and Ostern and Parnas (1932) state that the perfused frog's heart is slowed by adenyl triphosphate, muscle and yeast adenylic acid and adenosine, and even nucleic acid of plant or animal origin. They noted in addition that the heart does not respond so readily to a second dose. The "refractory state" persists for some time. Inosinic acid, adenine and the guanine derivatives on the other hand they found to be inactive. Wedd and Fenn (1933) obtained slowing of the turtle's excised auricle with adenosine and yeast

adenylic acid. Gillespie (1934) speaks of heart block in the perfused frog's heart with the following substances all of animal origin; adenyl triphosphate and inosine triphosphate, which he found the most active; muscle adenylic acid and inosinic acid which proved a little stronger than adenosine, while adenine and hypoxanthine were without effect. Flössner (1934) investigated a considerable number of nucleic acid derivatives and found that inosinic acid was active in slowing the heart, and that all the derivatives even down to the simple products such as adenine and guanine were active.

From all these observations it can be safely concluded that nucleic acid and its derivatives when they act depress the function of the specialised tissue of the heart. In some animals the action is emphasised upon the pacemaker, in others upon the auriculo-ventricular bundle. The only exception to this is the human heart. It seems possible in this instance that they have an excitant action upon the sinus and a depressant action upon the A. V. bundle.

When the individual substances are considered there seems general agreement that muscle adenylic acid is active, and yeast adenylic acid is relatively inactive. Gillespie holds that the addition of the labile and the stable phosphoric acid to adenosine increases the activity, and finds that adenyl triphosphate is the most potent substance. There is difficulty in reaching a definite conclusion about the activity of inosinic acid. This substance has been found by some observers to be active, by others inactive. There is a similar discrepancy in the results with the guanine series. Adenine is apparently a weakly active substance. The pyrimidine products have been scarcely investigated. Although there may be slight doubt about the most active substance of the adenyl group, it would be agreed by all that loss of the sugar molecule definitely deprives the substance of a great deal of its activity. A comparable loss of activity occurs if the amino-group is removed.

INFLUENCE UPON THE GENERAL BLOOD PRESSURE. One of the most interesting actions and certainly one of the most discussed, is the influence of these substances upon the general blood pressure. In 1929 Drury and Szent Györgyi reported that adenosine and muscle adenylic acid produced a fall in general blood pressure when they were introduced intravenously into the intact anesthetised dog or cat. Bennet and Drury (1931) amplified these findings, using in addition yeast adenylic acid, upon the dog, cat, rabbit and monkey. The cat proved to be the least susceptible of all the animals, but in the others 0.15 to 0.3 mgm. per kilo produced a fall of about 30 mm. Hg. The fall and the re-

turn to the preinjection level were abrupt except with yeast adenylic acid, when the return was relatively slow. The monkey, however, even with yeast adenylic acid did not exhibit the slow return nearly so readily as the other animals. Whatever substance or animal was used, the effect was short lived, and at the best only lasted a matter of 2 to 3 minutes. Massive doses naturally led to more pronounced and prolonged effects, chiefly on account of the extreme slowing of the heart which simultaneously occurred. The animal recovers from massive doses quite readily, and it is difficult to kill an anesthetised animal by these substances, especially if it is artificially respired. About the same time Zipf (1931a) reported that muscle and yeast adenylic acid produced a fall in anesthetised rabbits, cats and dogs and de Caro (1931) noted the same effect with adenylic acid. v. Euler (1932) found that guanylic acid had no action. Using the higher phosphorylised compounds Gillespie (1934) noted that adenosine triphosphate and inosine triphosphate produced a fall, preceded by a definite rise of blood pressure. Muscle adenylic acid and inosinic acid derived from these higher compounds gave the usual simple fall so that the rise is associated with the labile phosphate radicle. He found that adenine and guanine were without action. Flössner (1934) who has tested a considerable number of derivatives confirmed the activity of yeast and muscle adenylic acid, inosinic acid and adenosine, but found that the products such as guanylic acid, guanosine, adenine, guanine, inosine and xanthine also produced a fall in general blood pressure. These findings do not agree with those of Gillespie (1934) or Wolf (1905) who could not obtain any result in the anesthetised cat with uracil, cytosine, xanthine, guanine, adenine or thymine. Drury (1932b) found that cytidylic acid lowered the blood pressure in the intact anesthetised rabbit and Flössner (1934) obtained a similar result with the pyrimidine derivatives tested by him. The nucleic acids themselves appear to be active for Mendel, Underhill and White (1903) using the nucleic acid from wheat embryo obtained a fall of blood pressure in dogs and cats with relatively heavy doses and Goubau and Goethem (1913) using the nucleic acid from avian red cells report a similar activity. Very few results have been reported of the action of adenosine, yeast or muscle adenylic acid in man. Richards (1934) found the blood pressure to be unaffected in most experiments though a slight fall was occasionally noted. Wedd (1934) states that symptoms of a definite fall in blood pressure are often seen after injection of adenosine. Wolf and Heinsen (1934) state that in elderly subjects there is a primary increase followed by a short-lived decrease in blood pressure both with muscle adenylic acid and adenosine.

Though it is true that most of the active depressor substances simultaneously slow the rate of beating of the heart, this is not the main cause of the fall in blood pressure. Bennet and Drury (1931) noted a fall in blood pressure of the perfused hind leg of a dog. In addition there is no relationship between the fall in blood pressure and the degree of cardiac slowing. Yeast adenylic acid which has but little influence upon the heart rate, is a potent depressor substance. The blood pressure change appears to be due to the dilatation of vessels chiefly the arterioles. Marcou (1931) considers that the splanchnic vessels are chiefly concerned. Bennet and Drury (1931) were unable to detect any influence upon capillaries judged by the introduction of adenosine into the skin. Zipf and Giese (1933) observed the peripheral vessels under a microscope and state that the capillaries dilate as well as the smaller arterioles.

It is apparent that nucleic acid and many of its derivatives especially the adenyl series reduce the blood pressure in the intact animal owing to their capacity to dilate blood vessels. The activity of these different substances differs very considerably but most observers have found adenosine to be the most active. There is in fact a close correspondence between the activity of the substances as judged by the depressor action, and by their influence upon the specialised tissue of the heart. There is one important difference, yeast adenylic acid which all observers find to be relatively ineffective upon the specialised tissue of the heart, is a potent depressor substance.

Adenylic acid and raised blood pressure. Lindner and Rigler (1931) reported that in cases of arterio-sclerosis the adenylic acid content of blood was lower than normal and suggested that this might be the cause of the raised blood pressure in such cases. They tested however such a small number of cases that, considering the wide variations in the normal values, the observations are of little significance. Rothmann (1931) investigated the occurrence of adenylic acid in blood and bile. He found that the amount present in blood depended chiefly upon the number of red cells present. He determined the amount present in various diseases. His cases of raised blood pressure gave normal values.

Influence upon the vessels in different organs: The heart. Drury and Szent Györgyi (1929) measured the coronary flow in a dog's heart-lung preparation and found that adenosine brought about a considerable increase in flow, while Rigler and Schaumann (1930) and Lindner and Rigler (1931) found that it increased the flow in the rabbit's perfused heart. Wedd (1931) using the latter preparation, compared the action

of adenosine with yeast and muscle adenylic acid; adenosine proved the most active dilator, but there was little difference between the two acids. The manner in which some of the above experiments were carried out indicated that the increased flow was brought about by dilatation of the vessels and not by any influence upon rhythm or muscular contraction. Bennet and Drury (1931) tested some related substances on the perfused rabbit's heart and found that yeast and thymus nucleic acid and inosinic acid were uncertain in their action, but occasionally increased the flow. Using the cat's perfused heart they found that both adenosine and muscle adenylic acid were active. This result is interesting in view of the fact that they have a very small influence upon general blood pressure in the cat. The coronary flow of the guinea pig's perfused heart is increased by adenosine (Bennet and Drury, 1931). Wedd and Drury (1934) compared the effect of adenosine, muscle and yeast adenylic acid and yeast cytidylic acid upon the coronary flow in the intact dog using the Morawitz technique. Yeast adenylic acid proved the most potent but yeast cytidylic acid was found to be almost as effective. Drury (1932a) found that yeast guanylic acid had a slight constrictor effect upon the vessels and Wedd (1931) reported that adenine had a slight and guanine a very slight dilator effect. The results are in general those which would be expected when the action of the various substances upon the general blood pressure is considered.

The lungs. The pulmonary artery. Bennet and Drury (1931) found that adenosine constricted the vessels of the rabbit's lung perfused through the pulmonary artery. The doses necessary indicate that at the best it has a weak constrictor action. Gaddum and Holtz (1933) using a more refined method in the dog and the cat noted that small doses produced a dilatation, but larger doses gave a dilatation that was immediately followed by a constriction. The vessels of the cat were much more susceptible than those of the dog. They tested three substances, adenyl triphosphate obtained from voluntary muscle, muscle adenylic acid and adenosine. Adenyl triphosphate produced both the effects of dilatation and constriction much more easily than the other two substances. Gaddum and Holtz (1933) were of opinion that their observations allowed them to compare the action of adenosine and adenyl triphosphate. They found that the addition of the extra phosphate groups to the molecule of adenosine increased its activity more than 3 times.

The ear. Bennet and Drury (1931) observed with a microscope the small vessels of the intact rabbit's ear. They found that these were

dilated when adenosine was introduced intravenously into the other ear. They also noted that the addition of adenosine to the solution perfusing a rabbit's ear led to a considerable increase in flow.

The kidney. Drury and Szent Györgyi (1929) using the heart-lung-kidney preparation of a dog noted a constriction of the vessels with adenosine. Bennet and Drury (1931) using the rabbit's kidney perfused with Ringer or blood Ringer solution found that the reaction with adenosine varied from animal to animal. In some instances a dilatation was recorded, in others a constriction, or a constriction followed by a dilatation. A weak reaction only was obtained. Marcou (1932) noted a constriction of the vessels of the dog's kidney perfused with Locke's solution.

The spleen. Marcou (1932) found that the isolated perfused spleen is decreased in size by an injection of adenosine. This may be due to a vessel constriction or to the contraction of the splenic tissue.

THE MUSCULAR SYSTEM: *Skeletal muscle.* Drury and Szent Györgyi (1929) tested the influence of adenosine upon the amplitude of contraction of the cat's left tibialis anticus muscle, subjected to intermittent tetanisation. No effect was seen except when a very large dose was used when a transient diminution in amplitude occurred. It can be concluded that adenosine has no action, for the change seen with the large dose is due to the fall in general blood pressure which occurs and which in itself is sufficient (Florey and Szent Györgyi, 1929) to bring about a diminution in amplitude. Wedd and Fenn (1933) were unable to detect any influence of adenosine upon the oxygen consumption of frog's skeletal muscle.

The heart. Drury and Szent Györgyi (1929) were greatly impressed by the fact that the hearts of their experimental animals appeared to keep in such good condition when injections of adenosine and adenylic acid were being investigated. Lindner and Rigler (1931) also observed that if the rabbit's perfused heart was rendered hypodynamic by oxygen want, adenosine caused an increase in the height of contraction. Wedd (1931) also seemed to gain the impression that in the perfused rabbit's heart adenosine caused a more vigorous beating and Rothmann (1930) on rather slender evidence considered that the heart was strengthened by muscle adenylic acid.

It was quickly appreciated by all workers that the nucleic acid derivatives dilated the coronary arteries and that this might account for the apparent improvement in the beat owing to the more adequate nutrition of the heart muscle. Nevertheless the improvement seemed so definite

that several workers carried out observations on this question. Drury and Szent Györgyi (1929) undertook a detailed investigation on the influence of adenosine and muscle adenylic acid on the auricular and ventricular muscle of the dog's heart and this was followed by Wedd and Fenn (1933) on the mammalian and cold blooded cardiac muscle.

As far as the auricular muscle is concerned all these workers agree that the amplitude of contraction is decreased. This effect is so obvious that it can easily be seen with the naked eye in the intact dog's heart (Drury and Szent Györgyi, 1929), and it has been confirmed by myograph records. The reduction occurs both when the auricle is beating normally and when it is driven at a constant rate; it is brought about by both adenosine and muscle adenylic acid. Wedd and Fenn (1933) found the same effect with entire excised auricles of the turtle, guinea pig and rabbit or with auricular strips from dogs using both adenosine and yeast adenylic acid. There appears little doubt therefore that the amplitude of contraction of auricular muscle is decreased by nucleic acid derivatives. When the ventricular muscle is considered the matter is by no means so certain. No change can be detected by the eye in the contraction of the ventricle of the intact dog, and the right ventricular myogram remains unchanged when the heart is responding to rhythmic shocks liberated from a constant point on the right ventricle (Drury and Szent Györgyi, 1929). These observations suggest that there is no influence upon the strength of contraction of ventricular muscle. Wedd (1931) confirmed this observation with adenosine in the perfused rabbit's heart, but Wedd and Fenn (1933) noted that the amplitude of contraction of turtle ventricular strips was sometimes decreased by adenosine. In a single experiment with a dog's ventricular strip moreover they noted a decrease in amplitude. A little later Drury (1932a) found that when either yeast adenylic acid or yeast guanylic acid was injected into the perfusate of a rabbit's heart, there was a temporary decrease in the amplitude of the ventricular myogram followed by a definite increase. This increase persisted for a considerable time after the dilatant action of the adenylic acid had passed away and occurred with the guanylic acid which was shown to constrict the coronary vessels. As no such change was recorded with adenosine, Drury (1932a) came to the conclusion that the alteration in the amplitude of contraction must be connected with the phosphoric acid grouping, and he noted that sodium triphosphate produced a similar effect to the two acids. He considered that the beat therefore was influenced by the adenyl compounds acting on the coronary vessels and by the phosphate radicles upon the muscula-

ture. Gillespie (1934) was unwilling to accept this conception as he felt that adenyl triphosphate and muscle adenylic acid should be much more active than adenosine in producing heart block, and because Drury used doses of inorganic phosphates far outside the range of those used in the adenyl derivatives. The first criticism is scarcely valid for heart block is produced by an action on specialised tissue while Drury was considering an action upon muscle. The second reason is much more pertinent. Gillespie (1934) holds that the action upon the muscle is always depressant, and finds it with adenyl and inosine triphosphate, adenylic acid and inosinic acid and adenosine and that the increase in amplitude when it occurs is due always to the increase in coronary flow. It is difficult to accept Gillespie's conclusions as he has taken no account of the fact that a change in rate in itself has a definite influence upon the amplitude of contraction (Dale, 1930, 1933). This fact has not been sufficiently appreciated, and the results of those workers who have not controlled the cardiac rate cannot be accepted as real evidence that the change in amplitude is due to an action upon the muscle. Parnas and Ostern (1932) however obtained in the frog's perfused heart results similar to those obtained by Drury (1932 a) in the rabbit's heart. They found that adenosine tri-phosphate and occasionally muscle adenylic acid increased the strength of the beat, and that adenosine failed to do so; a result which was obtained by Lindner and Rigler (1931). Flössner (1934) found that some nucleic acid derivatives increased while others decreased the amplitude of contraction of the perfused frogs heart. As the matter stands at the moment it is probably correct to conclude that the increase in amplitude of the ventricular contraction is on most occasions due to the increased coronary circulation. On the other hand, in the perfused rabbit's heart at any rate, yeast guanylic acid which constricts the coronary arteries, gives a prolonged increase in amplitude of contraction. It is difficult in face of this observation to consider that the alteration in coronary supply is the sole factor. Further observations especially those employing adenyl triphosphate and muscle adenylic acid are very desirable. A few observations have been made by Wedd and Fenn (1933) on the influence of adenosine on the oxygen consumption of frog's cardiac muscle. Their results are inconclusive but in some experiments there were definite indications of an increased oxygen consumption when adenosine was added. They are not inclined to stress their results and feel that the improvement in beat is adequately explained by the increase in oxygen supply resulting from the vasodilatation.

Drury and Szent Györgyi (1929) and Wedd and Fenn (1933) have determined the influence of adenosine, yeast and muscle adenylic acid upon the refractory period of auricular and ventricular muscle. They both find that the refractory period of auricular muscle is reduced by about 20 per cent. Wedd and Fenn (1933) noted also that the duration of auricular systole was shortened by adenosine. All these observations are in accord with the decrease in amplitude of contraction already noted. The refractory period of ventricular muscle on the other hand was found by these same observers to remain undisturbed, after the injection of adenosine and muscle adenylic acid. This observation is consistent with the absence of any change in the ventricular myogram reported by Drury and Szent Györgyi (1929).

Conduction in auricular muscle was investigated by Drury and Szent Györgyi (1929), who found that if the auricle was beating slowly there was no influence upon normal conduction, depressant or otherwise. If, however, the auricle was driven at a high rate so that each contraction occurred immediately after the refractory period of the preceding had terminated, the slow conduction thus occasioned was improved by adenosine probably owing to its capacity to shorten the length of the refractory period. No evidence could be obtained of any influence upon conduction in ventricular muscle.

A peculiar effect of these substances was noted by Drury and Szent Györgyi (1929). Auricular flutter and fibrillation could be produced by electrical stimulation extremely readily when the heart was under the influence of adenosine. On the other hand, if the same substance was introduced intravenously into a dog in which experimentally produced flutter or fibrillation of the heart was present, the disordered action was consistently stopped. Both these results can be obtained during vagal stimulation, and they have been ascribed to certain characteristics of the muscle which are associated with the shortened refractory period produced by the stimulation (Lewis, 1925). It is reasonable to conclude that adenosine by its capacity to shorten the refractory period produces similar conditions. Honey, Ritchie and Thomson (1930) injected adenosine into human subjects with auricular fibrillation but could detect no influence upon the disordered action of the auricle. Similar negative results were obtained by Fahrenkamp and Schneider (1930), Rothmann (1930) and Jezer, Oppenheimer and Schwartz (1933). The last authors found in addition that adenosine had no influence upon paroxysmal tachycardia. The dose used was sufficient to produce definite conduction disturbances in the auriculo-ventricular bundle.

The form of the electrocardiogram. The electrocardiogram is but little changed by the adenyl compounds. Honey, Ritchie and Thomson (1930) report a slight increase in the height of the P wave and of the T deflection and even alternation, while Rothmann (1930) observed that the R wave was sometimes increased in height. Hartmann (1932) found on the other hand that the R deflection and the T wave were decreased and the latter was occasionally reversed. Similar observations have been reported by Van der Velden (1932) and Richards (1934). Jezer, Oppenheimer and Schwartz (1933) made no comments on this point, and their records show no material change. All the above observations relate to the human subject. Drury and Szent Györgyi (1929) noted that the contour of the P wave and the direction of the T wave was changed in the dog with adenosine. In the guinea pig the form is apparently undisturbed. The alteration in heart rate which occurs though it may explain some, will not explain all of the recorded examples. The changes, which are at the best slight and variable, do not suggest that the substances have any profound effect upon the ventricular muscle.

It may be concluded that the action upon the heart is definitely depressant as far as the auricular muscle is concerned. This holds for all the derivatives which have been tested, and is supported by the changes in the refractory period and the duration of systole which have been observed. The action upon the ventricle is still a matter of dispute. While some observers hold that the substances all act as depressants others find that they improve the beat or have no specific action. The observations upon the refractory period suggest that the drug has no influence at all upon the ventricle. The increased amplitude of contraction which occurs may well be due to the coronary dilatation which is simultaneously produced. Against this there is the observation with guanylic acid; a substance which improves the beat and at the same time decreases the coronary flow. At the moment therefore no final conclusions can be reached as to whether the nucleic acid derivatives, even the higher phosphorylised substances such as adenyl triphosphate, muscle or yeast adenylic acid, and yeast guanylic acid have a specific action upon the ventricular muscle. However most workers are agreed that hearts of experimental animals whether perfused or in the intact animal remain in very good condition when subjected to injections of these substances. Moreover, it is difficult for workers to convince themselves that the improvement is always due to the action upon the coronary vessels. It is a problem of great interest and importance which the future must decide.

Intestine. Drury and Szent Györgyi (1929) reported that adenosine inhibited the intestinal movements of the intact anesthetised cat. In addition the rabbit's isolated intestine suspended in Tyrode's solution was inhibited when the concentration in the solution was about 1–50,000. Zipf (1931a) in his experiments with yeast and muscle adenylic acid showed them to have a similar activity. v. Euler and Gaddum (1931) found adenosine to have the action noted by Drury and Szent Györgyi. Flössner (1934) has tested a number of nucleic acid derivatives and finds that they all (including the guanine series) inhibit the isolated intestine of the rabbit. Vicine and the derivative, 4, 5 diamino 2, 6 dioxypyrimidine both stimulate the guinea pig's intestine. v. Euler (1932) found that yeast adenylic acid inhibited the rabbit's intestine but that guanylic acid was without action.

Barsoum and Gaddum (1935) have developed a delicate test for the adenosine equivalent of crude extracts which makes use of the inhibitory activity of the adenyl compounds upon intestinal muscle.

The bronchioles and trachea. Bennet and Drury (1931) found that adenosine and adenylic acid both dilated the bronchioles. The effect was observed more clearly if the bronchioles had been constricted by a small dose of histamine. The trachea, constricted by pilocarpine, was also dilated by these two substances.

The uterus. The action of yeast and muscle adenylic acid upon the isolated guinea pig's uterus was first noted by Zipf (1931a) who found that both had a stimulating action. This was followed by the observations of Bennet and Drury (1931) using adenosine, yeast and muscle adenylic acid; all three substances stimulated the uterus, adenosine being the most active. In the same year v. Euler and Gaddum (1931) also showed the stimulating action of adenosine. The only extensive series of observations which have been made are those of Deuticke (1932). He used muscle and yeast adenylic acid, muscle inosinic acid, adenosine triphosphoric acid, and the adenine nucleotide obtained from heart muscle ("Herznucleotid" of Embden). All the substances stimulated the guinea pig's uterus except muscle inosinic acid which proved inactive. In contradistinction to the observations of Bennet and Drury (1931) he found that the adenosine was weaker than muscle and yeast adenylic acid, and that these substances were weaker than adenosine triphosphate and the heart nucleotide. The activity became greater as the number of phosphoric acid radicles was increased. After the addition of a small dose the uterus does not respond to additional doses; it is in a sense "refractory." Flössner (1934) has tested a number

of derivatives upon the uterus of the guinea pig and the cat and finds that adenosine is more active than the adenylic acids. In addition inosinic acid appears to be weakly active, and the same can be said of the many other derivatives tested by him. v. Euler (1932) found that yeast adenylic acid constricted the guinea pig's uterus but that guanylic acid was inactive. Flössner (1934) found that the latter substance constricted and Drury (1932a) that it relaxed the guinea pig's uterus.

Influence upon secretion. The observations which have been made are few, but there is no indication that the substances have any influence upon secretion. No increase in salivary secretion has been reported, and considering the number of opportunities which must have been available for such an observation, it may safely be concluded that the substances are without influence. Bennet and Drury (1931) did not obtain any evidence of increased biliary secretion in the guinea pig with adenosine. Vandolan (1933) found that muscle adenylic acid had no influence upon gastric secretion. Adenosine (Drury and Szent Gyorgyi, 1929) had no influence upon the secretion of urine unless it was given in heavy doses. The decreased flow of urine seen was probably due to the decrease of the blood flow to the kidney which simultaneously occurred.

Leucocytosis. For some years sporadic observations have been made upon the influence of nucleic acid upon the leucocytes. In 1897 Ames reported his observations on the leucocytosis produced by nucleinic acid, and since that time a series of clinical and surgical papers has appeared dealing with the same subject (de Paoli and Calisti, 1912; Jacobi, 1921; Medwin Gardner, 1924; Daubenton 1925). The increase in white cells in many cases was not great and as the normal range of fluctuation is about 100 per cent, the results cannot be considered as very significant. In 1913 Ewald showed that yeast and thymus nucleic acids, xanthine and guanine produced a leucocytosis in dogs but that uric acid was inactive. Neymann (1917) found that a definite leucocytosis was produced by sodium nucleinate in the human subject which lasted for 1 to 2 days. Fox and Lynch (1917) using dogs described a leucopenia immediately after the injection of sodium nucleinate and a subsequent definite leucocytosis which concerned chiefly the polymorphonuclear leucocytes. Larsell, Nokes and Phillips (1926) reported a clinical and experimental study on the influence of nucleic acid from fowls' red cells. They found that hemopoiesis was stimulated in the rabbit, and temporarily stimulated in selected cases of anemia in the human subject. Doan, Zerfas, Warren and Ames (1928) carried out an extensive series of observations upon the leucopenia and leucocy-

tosis produced by sodium nucleinate from yeast, and found that the same effects were produced by the yeast nucleotides, adenylic acid and guanylic acid. Reznikoff (1929) noted that yeast and thymus nucleic acid, and in addition the purine bases, adenine sulphate and guanine hydrochloride, produce a leucopenia followed by a leucocytosis in rabbits.

Bennet and Drury (1931) using adenosine, muscle and yeast adenylic acid found a leucopenia followed by a leucocytosis, and the increase was made up in great part by the polymorphonuclear leucocytes and lasted for 26 to 48 hours. They also found that if they instilled the substances into the rabbit's eye a bead of pus, consisting almost entirely of polymorphonuclear leucocytes, collected at the inner canthus. On the other hand Drury (1932a) could get no evidence of leucocytic accumulation in the rabbit's eye when yeast guanylic acid was instilled, or a general leucocytosis when it was injected (Drury 1934), although in the hands of Doan et al. (1928) it produced a definite leucocytosis.

Additional evidence of the capacity of nucleic acid derivatives to increase the number of leucocytes is afforded by the observations upon the human subject suffering from malignant neutropenia. Reznikoff (1931) reported an increase in the number of polymorphonuclear leucocytes after intravenous injections of the purine bases adenine and guanine in the human subject, and Jackson, Parker, Rinehart and Taylor (1931) using the crude nucleotides from an alkaline hydrolysis of yeast obtained the same result. The fact that several different nucleotides can now be obtained in the pure state offers an opportunity to determine the clinical value of these different nucleotides.

It seems definite therefore that nucleic acid and its derivatives even down to the simple products such as adenine and guanine are capable of producing a leucopenia followed by a polymorphonuclear leucocytosis. The manner in which they act must be a subject of conjecture. The effect seen in the rabbit's eye suggests that they act as chemiotactic substances and Doan, Zerfas, Warren and Ames (1928) came to the same conclusion. The leucopenia according to their observations is due to the accumulation of the polymorphonuclear cells in the spleen.

THE OCCURRENCE OF ADENYLIC ACID IN THE ANIMAL BODY. The distribution of adenylic acid in the body has been determined both by chemical isolation and biological tests. It has been isolated by Jackson (1923–1924) from the blood, Embden and Zimmermann (1927) from skeletal muscle, Pohle (1929) from the heart and brain, and more recently by Embden and Deuticke (1930) from the kidney. Zipf (1931a) has isolated it from blood, Calvary (1930) from the urine and Rothmann (1931) from the bile.

Bennet and Drury (1931) relied upon the biological test. They held that the duration of the heart block in the guinea pig provided a simple method of testing the adenosine equivalent of adenylic acid contained in a crude extract. They found that while adenylic acid was distributed generally throughout the body, it was much more plentiful in some tissues than in others. Heart muscle and striated muscle contain the most, while skin and lungs contain very little. The testis and ovary have considerably less than muscle. Recent unpublished work by Drury has shown that adenyl triphosphate produces heart block in the guinea pig, and is slightly more active than muscle adenylic acid. The results of Bennet and Drury (1931) can no longer (as they stated) be held to represent the distribution of adenylic acid, for the extracts may have contained adenyl triphosphate in addition. The observations however probably indicate accurately the comparative distribution. Parnas and Ostern (1932) used the frog's heart to determine the distribution. The adenosine equivalent obtained by them for the different organ extracts was about 2 times greater than that found by Bennet and Drury (1931). Their values must be considered the more accurate as they agree more closely with the chemical estimations. The difference in the results is probably due to the method of preparing the crude extracts. However the comparative distribution throughout the body agrees with that found by Bennet and Drury. The recorded observations indicate that the adenyl compounds are widely distributed. They are probably constituents of all animal cells. The chief point of interest at the moment is that they appear to be present in considerable amount in tissues in which histamine is relatively scanty, and vice versa.

THE NUCLEIC ACID DERIVATIVES AND TISSUE EXTRACTS. It would be of no interest, except from a historical point of view, to consider the part that the adenyl compounds have played in the vascular reactions which have resulted from the injection of tissue extracts. It is obvious in the light of our present knowledge that they must have been present in many of the extracts tested, and have added their effects to the other dilator substances present. There are, however, many other dilator substances present in tissue extracts some of which remain unidentified even today. There are at least five or six such substances which may occur in tissue extracts. There is histamine, the characteristics of which have been fully worked out and have been reviewed by Best and McHenry (1931). There are choline and the choline esters, and the substances allied to adenosine which form the subject of this review. In the pancreas Frey, Kraut and Schultz (1930) have evidence for a

depressor substance, Kallikrein. v. Euler and Gaddum (1931) and Gaddum and Schild (1935) are investigating another depressor substance obtained from the intestine. This may be the same or a similar substance to that which Felix and his co-workers (1933) are attempting to identify. In addition Major (1930) and his co-workers have investigated a depressor substance from brain extracts. Weber, Nanninger and Major (1933) have recently stated that they have obtained this substance in crystalline form; if this should prove correct it should be possible to decide whether this substance is the same as any of those under investigation by other workers. There is much difficulty in disentangling the evidence as to the nature of the substances concerned and of deciding if they are identical or not. At any rate it is certain that several different dilator substances are present in tissue extracts, the physiological or pathological import of which it is difficult to estimate at the present time. The review by Dale (1933) is an excellent statement of the position as it now stands. The reason for emphasizing the number of possible dilator substances which may be present in crude tissue extracts is that they have been and are being extensively used in hospital practice, and the dilator (or beneficial) action which is said to occur has been ascribed to the adenyl compounds they contain. Upon this subject there is already an extensive literature. The results which are being obtained with such extracts are not very conclusive, and if they were conclusive it would be difficult to ascribe the beneficial action to adenyl compounds when it is known that other dilator substances, some of which are unidentified may be present.

Table 1 indicates the commercial substances which have been tested for the presence of adenyl compounds. The references cited may prove useful to anyone interested in the clinical aspect of the question.

Cozymase. H. v. Euler (1930) and his co-workers found that the cozymase obtained from yeast and from several animal organs had the probable constitution of an adenylic acid. Cozymase is however apparently not identical with any of the adenylic acids which have been isolated from muscle and yeast, and it belongs to the same group as inosinic acid and muscle adenylic acid (Myrbäck and H. v. Euler, 1930). v. Euler and Gaddum (1931) had the opportunity of testing a highly purified solution of cozymase and found that it caused a fall in blood pressure in atropinized rabbits. It stimulated the guinea pig's isolated uterus and inhibited the rabbit's isolated intestine. The preparation of cozymase appeared to have about the same activity as muscle adenylic acid. Cozymase (Sym, Nilsson and v. Euler, 1930) apparently

exists in various organs in the animal body, and its distribution is very similar to that of adenylic acid. It may therefore be one of the depressor substances present in organ extracts.

BIOLOGICAL TESTS FOR ADENYL COMPOUNDS. One of the main difficulties in the way of further progress is that the biological tests at present available require large amounts of adenyl compounds. The test used by Bennet and Drury (1931)—namely, heart block in the guinea

TABLE 1

PREPARATION	ORIGIN	ADENYL COMPOUNDS	REFERENCES
Eutonon	Liver	Absent	Parnas and Ostern, 1932. Joos, 1933. Heinsen, 1934
		Present	Hochrein and Keller, 1931
Angioxyl	Pancreas	Absent	Parnas and Ostern, 1932
Lacarnol	Muscle	Present	Lindner and Rigler, 1931. Hochrein and Keller, 1931. Singer, 1931. Schittenhelm, 1931. Thanhauser, 1931. Krayer, 1932. Joos, 1933
Padutin (Kallikrein)	Pancreas	Absent	Hochrein and Keller, 1931. Joos, 1933
Schwarzmann's extract	Muscle	Absent	Schwarzmann, 1933
Myoston and Myol	Muscle	Present	Parnas and Ostern, 1932. Joos, 1933
Hormocardiol	Sinus of heart	Present	Hochrein and Keller, 1931
Campolon	Liver	Absent	Heinsen, 1933. Wolf, 1934
Hepatopson	Liver	Absent	Wolf, 1934
		Present	Heinsen, 1933
Degewop	Liver	Occasionally present	Wolf, 1934
		Present	Heinsen, 1933
Hepatrat	Liver	Absent	Wolf, 1934

pig's heart, even if the animal is rendered as sensitive as possible, requires the equivalent of 20γ of adenosine to produce a definite effect. The advantage of the guinea pig's heart test lies in the fact that it is apparently specific for adenyl compounds and can be used with crude extracts as it is not influenced by the presence of the many varied substances present in tissue extracts (Bennet and Drury, 1931). Ostern and Parnas (1932) using the perfused frog's heart, find that they can obtain

effects with about 0.4γ of adenosine. Gaddum and Schild (1935) have obtained inconstant results with this test. Lately Barsoum and Gaddum (1935) using the fowl's intestine have developed a method which will estimate the equivalent of 0.2–0.5γ of adenosine present in crude blood or tissue extracts. This seems the most satisfactory test available at the moment; it offers an opportunity for investigating changes in adenosine equivalent in blood in experimental conditions such as reactive hyperemia, etc. This test, however, may not prove sufficiently sensitive for the purpose and there is still definite need of one more delicate.

THE POTENCY OF THE VARIOUS PRODUCTS. In considering the potency of the different derivatives it is important to remember that many different reactions are brought about by these substances, and that the order of potency as judged by one reaction is not necessarily that judged by another. It must also be remembered that the various substances tested have been almost without exception derived from many different sources. Gillespie (1934) alone has made a series of products derived from one mother substance, adenosine triphosphate, and has tested their relative potency. These two considerations must certainly come into prominence when the detailed investigation of potency is undertaken. At the moment, however, it is only possible to discuss the potency in very general terms, and the order, with one or two major exceptions, is the same whether it is tested on the heart, blood pressure, intestine or uterus. The most active of the substances can only be considered fairly potent. They have no potency of the order of histamine or acetyl choline but rather that of free choline. Their influence is only short lived for the body quickly converts the active products into relatively inactive substances. This is perhaps the reason why subcutaneous injections have not on the whole been successful. The action upon the specialised tissue of the heart has been most carefully worked out, and it is agreed by most workers that adenosine weight for weight is the most active substance. In the intact guinea pig of about 200 grams, a definite reaction is obtained with 0.1 mgm. of adenosine, and 0.2 mgm. of muscle adenylic acid. Yeast adenylic acid is very inactive, 9.0 mgm. being required to give an effect of the degree produced by 0.1 mgm. of adenosine. The potency of muscle adenylic acid is definitely reduced if not lost altogether when the amino group is split off, for inosinic acid in doses 12 times that of adenosine proved inactive (Bennet and Drury). Removal of the sugar molecule also reduces the potency, for adenine in a dose 8 times greater than that of

adenosine produces a considerably smaller effect. All the members of the guanine series appear to be inactive. Cytidylic acid (Drury, 1932b) has no action upon the guinea pig but slows the rat's heart. Little difference in potency is noted if the substances are tested upon the perfused rabbit's heart. Wedd (1931) found adenosine was more active than muscle adenylic acid and that both slowed the heart considerably. Yeast adenylic acid was practically ineffective. Gillespie (1934) on the other hand states the higher phosphorylised compound, muscle adenyl triphosphate, is more active in producing heart block in the rabbit's perfused heart than muscle adenylic acid, and this latter substance more active than adenosine, inosine triphosphate and inosinic acid being weakly active. Drury (1934) also found that adenyl triphosphate was slightly more active than adenosine in the intact guinea pig and in this respect the observations agree with those of Gillespie (1934). The nucleic acids (Drury and Szent Györgyi, 1929) had no influence. Judged by their action on the frog's heart, the same relative potency holds. Gillespie (1934) noted however that inosine triphosphate and inosinic acid were weakly active and Flössner (1934) states that inosinic acid is definitely active. This does not agree with the findings of Ostern and Parnas (1932) who found inosinic acid to be inactive. Nucleic acid in their hands, whether of animal or plant origin was very weakly active and adenine and guanine were inactive. Flössner (1934) found that members of the guanine series had a potency which did not differ very much from that of the adenyl compounds, and that the pyrimidine derivatives tested by him were also active.

If the potency is judged by the capacity of the substance to dilate the blood vessels, to inhibit the intestine or to constrict the uterus there is a general agreement with the results of the observations upon the heart. There are one or two important differences. Yeast adenylic acid dilates the blood vessels and has an activity similar to that of muscle adenylic acid though it has no action upon the rhythm of the heart. Cytidylic acid (Wedd and Drury, 1934) dilates the coronary vessels, and compares very favourably with muscle adenylic acid. These two substances judged by their vessel action must be considered as definitely active substances though they are relatively ineffective upon the heart.

Taking all the recorded observations together there seems little doubt that as far as the influence upon the heart, blood vessels, intestine and uterus is concerned the nucleic acids are in themselves relatively inactive and that the greatest activity is met with in the adenyl series of

derivatives. In some reactions the addition of the stable and labile phosphate molecules to adenosine appears to increase the activity definitely, but this increase is not always found. Be that as it may adenyl triphosphate, muscle, yeast adenylic acid and adenosine are the most active members of the group. It is still a matter of dispute whether the substances are active after deamination, or removal of the sugar molecule. The decrease in activity is very great and large doses have to be employed to obtain a result. The question of purity must arise for if there were a trace of the higher compound remaining, the positive result might well be due to it. At any rate deaminated compounds, and compounds from which the sugar has been removed, have been tested on many occasions and there is no uniformity in the results obtained. At the moment, therefore, it is impossible to come to a definite conclusion whether deamination or removal of the sugar molecule deprives the adenyl compounds of their activity, completely or only in part.

The guanine series has been so little tested that no definite conclusions can be drawn. There is however no uniformity in the results obtained, some observers finding them active, and others without action. They are at the best relatively inactive. Very few observations have been made upon the pyrimidine series. There are indications that definitely active substances might be found in this series as far as the action upon the blood vessels is concerned.

When the question of the leucocytic action is considered it is obvious that an entirely different order of potency will be found. According to Doan et al. there is little difference between the action of yeast adenylic acid and yeast guanylic acid and Ewald and Reznikoff found that the nucleic acids as well as adenine, guanine and xanthine are quite active in this respect. It can only be concluded from this striking difference that the substances exert their leucocytic effect in a manner which is entirely different from that which gives rise to the depressor action.

Before concluding this review there are one or two problems of general physiological and pathological interest which must be mentioned. When biologically active substances of natural origin are isolated it is simple enough to establish their different activities but it is extremely difficult to find their place in physiological or pathological reactions. There are however certain reactions in which it might be imagined that the adenyl compounds would play a part and it is proposed to deal very briefly with these problems. To many workers this possibility will represent the main spur for further work and experi-

ment. There can be no objection against drawing attention to these problems even if at the moment there seems little or nothing[1] to justify it, if it is clearly understood that the considerations are speculative.

THE TOXICITY OF FRESH DEFIBRINATED BLOOD. *"Frühgift."* In 1920 Freund described the toxic action of fresh defibrinated blood; which was called by him the "Frühgift." If such blood was injected into animals a severe "shock" often resulted, with fall in blood pressure and temperature. He found that the responsible substances which rapidly disappeared dilated the skin vessels of the dog, inhibited the intestine of the rabbit and constricted the uterus of the guinea pig.

In 1930 Zipf and Wagenfeld reinvestigated this toxic action. They found that the intravenous injection of fresh defibrinated blood, whether obtained from a similar or the same animal, led to a fatal shock with low blood pressure and subnormal temperature. A disordered cardiac action was sometimes observed but the fall in blood pressure was determined by a dilator effect on the blood vessels, and was seen after atropinisation and vagal section. They were of the opinion that the substance responsible for the effects witnessed was neither histamine, choline nor acetyl choline. Moreover they noted that the toxicity rapidly disappeared from the blood, and that filtrates of the blood were active.

Later Zipf (1931a) reported that the extracts of many animal tissues produced toxic effects similar to that of fresh defibrinated blood. Adenine and phosphoric acid were isolated from the active extracts. From these and other chemical considerations Zipf came to the conclusion that the substance responsible for the Frühgift was adenylic acid and that this arose from the breakdown of the cellular elements of the blood.

His later papers have emphasised the similarity of the physical and chemical qualities, as well as of the pharmacological effects, of adenylic acid and the substance responsible for the toxic action of fresh defibrinated blood. Zipf is now definitely of the opinion that adenylic acid is the substance responsible for the toxic action (Frühgift). This conception has received support quite recently from the work of Barsoum and Gaddum (1935) who find that the adenosine equivalent of blood increases upon standing for the first few hours.

[1] Zipf (1932) in a brief communication has stated that the amount of adenosine like substances is increased in many of the physiological and pathological reactions which will be mentioned. Unfortunately no detailed communication has been published by him on this important aspect and until such is available, it is impossible to evaluate his findings.

REACTIVE HYPEREMIA. Zipf (1931b) suggested that the liberation of adenyl compounds might well account for the phenomenon of "reactive hyperemia" which is seen after occlusion of the vessel supplying a tissue. In a brief communication, he states that the adenosine equivalent is increased after occluding a vessel to a limb (1932). The work of Lewis and Grant (1926) upon man indicates that the reaction is due to the accumulation of substances which are the products of metabolism during the period of circulatory arrest. They are of low diffusibility and are probably confined to the tissue spaces rather than to the vessel walls. These authors do not feel that any actively diffusible substance can be responsible for the long lasting reactions which are displayed. The work of Goldblatt (1926) upon dogs also suggests that the reaction is of metabolic origin. Although both these series of observations were carried out before the physiological activity of the adenyl compounds was appreciated, there is no clue in either as to whether the adenyl compounds can be the responsible substances or not. The fact that the adenyl compounds are readily diffusible as pure substances is no evidence that they must be such when liberated in the tissues during vessel occlusion. At the moment therefore actual proof that the adenyl compounds are responsible substances is wanting, but there is no evidence against such a possibility. A proof of such an influence would be interesting as Lewis and Grant have noted that the degree of vasodilatation after long occlusion is not dissimilar to that produced by strenuous and brief exercise of the muscles and the duration of the reactions is the same. The hyperemia produced by heat is accompanied by an increase in the amount of adenosine like substance according to Zipf (1932). Recently Barsoum and Gaddum (1935b) have shown that the blood coming from a limb during reactive hyperemia contains considerably more histamine than normally. They consider that histamine is the cause of some of the phenomena of vasodilatation. The capillary dilatation may well be due to the presence of the histamine, but it is difficult to see how this can cause the arteriolar dilatation which occurs.

VASODILATATION ACCOMPANYING MUSCULAR ACTIVITY. There is no doubt that adenyl compounds of more or less complexity exist in large amounts in heart and skeletal muscle and it must have occurred to all workers with these substances that they might be the important substances for regulating the blood flow through the resting and active organs. Zipf (1931b) and Rigler (1932) expressed this opinion quite early. This view gains support from the work on muscle contraction.

It now seems to be established, as a result of investigations by Embden and his collaborators, Parnas and Ostern and other workers, that the process of muscular contraction is associated with the breakdown of adenylic acid. Two main changes occur in the adenylic acid complex—the pyrophosphoric acid is liberated and the inosinic acid is produced by deamination with a liberation of ammonia. At the moment, however, actual direct experimental proof that these changes can play any part in the dilatation following muscular activity is wanting. Rigler's (1932) observation that the activity of the frog's muscle after atropinisation and poisoning with iodo-acetates (which is no longer therefore liberating lactic acid) is still accompanied by a vasodilatation which is similar to that produced by adenosine is suggestive. It is evidence that the vasodilatation is not produced by lactic acid or acetyl choline. This is interesting for the effects of lactic acid and carbon dioxide have never seemed quite sufficient to account for the enormous increase in flow during and following muscular activity. Rigler (1932) has suggested that the adenyl complex may be held firmly in the muscle protoplasm and that some of the free adenylic acid liberated in contraction may escape and so produce vasodilatation. It may be that the more complex compounds are relatively poor vasodilators. Gillespie (1934) states that the dilator action is increased slightly if the labile phosphoric acid is removed and still more if the stable phosphoric acid is removed. Removal of the pyrophosphoric acid during muscle activity may therefore unmask a more active dilator substance. Dale (1933) states on the other hand that free adenylic acid is not more but somewhat less active than adenyl triphosphate. The production of inosinic acid by deamination during the contraction process cannot play any appreciable part in the vessel dilatation for this substance is a very weak dilator substance. Zipf (1932) states that the amount of adenosine-like substances is increased by tetanising a muscle.

It must be admitted, therefore, that there is at the moment no direct proof that the adenyl compounds play any part in this vasodilatation. On the other hand it seems unlikely that the presence of large amounts of these fairly potent vasodilator substances in organs which show great dilatation during activity is without significance.

THE RESPONSE OF A TISSUE TO INJURY. Lewis and his co-workers (1927) have investigated the reaction of the skin to injury and have come to the conclusion that the visible response—the triple response—is produced by a substance akin to histamine, for injections of this substance reproduce the effects in great detail. The adenyl compounds

probably play no part in this local reaction as they are present in the skin in very small amounts and actual injection of adenosine into the skin leads to none of the effects seen after injury (Bennet and Drury, 1931). Lewis (1927) found in addition that a flushing of the cheeks similar to that produced by a subcutaneous injection of histamine, occurred when the skin of a suitable subject was injured; the H substance being apparently released into the circulation. Kalk (1929) found that a similar injury evoked a secretion of gastric juice. Lewis' observations are not incompatible with the liberation of adenyl substances into the blood stream since Richards (1934) has shown that the cheek temperature may rise after the injection of adenosine. On the other hand Kalk's observations are definitely against it for adenosine does not (Vandolan, 1933) increase gastric secretion. Therefore, it would seem reasonable to conclude that a substance of histamine-like character is liberated when the skin is injured. This histamine-like substance cannot be responsible for a reaction severe enough to be classed as inflammatory, for Grant and Wood (1928) have shown in many ways that histamine has no appreciable power to cause emigration of leucocytes from the blood vessels, a reaction which is an essential part of inflammation. Wolf (1923) and Findlay (1928) on the other hand came to the conclusion that histamine was a positively chemiotactic substance. It is still open to question whether histamine is a leucocytic substance or not, and till this is settled the part played by histamine in inflammatory reactions cannot be decided. On the other hand the adenyl compounds not only produce vasodilatation but give rise to a local and general leucocytosis and it is in this last aspect of inflammation especially that they may well play a part. In support of this Zipf states briefly (1932) that the amount of adenosine like substances in the rabbit's ear is increased during inflammation.

It may well be that a combination of histamine and adenyl compounds may be sufficient to account for the whole of the vessel reaction and leucocytosis seen after injury. Even so it would be rash to assume that they are the only substances responsible for the complex response of inflammation.

Shock. A few years ago it was widely accepted that in traumatic shock the circulatory failure was due to the absorption of toxic products from the damaged tissues. This conclusion was reached by Bayliss (1919) and Bayliss and Cannon (1919) as a result of an experimental study of the effect of severe muscular damage. They considered moreover that clinical data obtained during the war supported this view.

At this time Dale (1919) and his co-workers showed how closely the effects produced by large doses of histamine in the cat and dog resembled the symptoms and signs recorded in certain cases of wound shock. It was only natural to link these two series of observations, and for many to conclude that histamine was the substance released from damaged tissue which gave rise to the condition of shock. It is only fair to note that the above workers never explicitly suggested that the toxic substance was histamine.

Later observations however have failed to support the conclusions reached by Bayliss and Cannon. The work of Smith (1928) questioned their conclusions and Blalock (1930) showed that the "shock" might be due to the effusion of blood into the damaged tissues, and not to an absorption of a toxic substance from them. Simonart (1930) on the other hand considers that the phenomenon is a result of nervous effects. There is therefore a definite doubt whether a circulating toxic substance is responsible for the shock produced by muscle injury. Although the toxic theory of shock is by no means so secure as it seemed in 1919, it is perhaps a little too early to discard it completely.

If the circulation of a toxic substance plays any part in traumatic shock, the adenosine series must be considered. "Traumatic shock" is most severe in extensive muscle damage, and the adenyl compounds are plentiful in this tissue while histamine is only present in small amount. König (1930) stressed the possibility of a substance identical with "Frühgift" being liberated during surgical operations and producing harmful results. Zipf (1931a) discussed the possibility of adenylic acid liberation during surgical operation, burns, x-rays and ultra violet ray therapy, and the shock which may accompany these procedures. Bennet and Drury (1931) sought for evidence of the release of adenosine-like substances after injury. They obtained reasonable evidence of the release of adenosine like substances from cardiac muscle by burning a rabbit's heart and leading the perfusate immediately to a guinea pig's heart. The guinea pig's heart showed disturbances which corresponded to those produced by adenosine and histamine combined. They also attempted to show the release of these substances in an intact anesthetised guinea pig, by scalding the lower portion of the animal and allowing its heart to receive blood from this region only, whereupon it developed changes similar to those seen after injection of adenosine. The latter experiments, however, were not very satisfactory and too much reliance cannot be put upon the results. Nevertheless the two series of observations combined give a reasonable support for the idea

that adenyl compounds are liberated after tissue damage. Whether they play any part in the production of the phenomenon of shock cannot be decided until the condition of traumatic shock has been more fully worked out. Zipf (1932) states that in traumatic shock, and in burns of the second degree, the amount of adenosine-like substances is increased.

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